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EFFECT OF AIR TEMPERATURE ON THE CONCENTRATION OF CERTAIN VIRUSES IN CABBAGE[1]

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INTRODUCTION

The crucifer viruses appear to belong to two distinct groups (*5*).[3] One group, represented by *turnip virus* 1 Hoggan and Johnson, contains cabbage virus A (*12*), the cabbage black ring virus (*10*), the cabbage ring necrosis virus (*3*), Tompkins' turnip virus (*9*), Hoggan and Johnson's turnip virus (*2*), turnip viruses described by LeBeau and Walker (*4*), and probably others (*5*). The second group, represented by *cauliflower virus* 1 Tompkins, contains cabbage virus B (*12*), the cauliflower mosaic virus (*8*), Tompkins' Chinese cabbage virus (*11*), and the broccoli virus of Caldwell and Prentice (*1*). Viruses belonging to the first group are favored by relatively high air temperatures, those of the second group by relatively low air temperatures (*5*). Symptoms produced by members of each group tend to become masked at unfavorable temperatures. When a virus of one group occurs in the host plant together with a virus of the other group, the resulting disease reaction is more severe than that of either virus alone, especially at high temperatures.

The present paper is a report of studies on the effect of air temperature and day length on the concentration of cabbage virus A, a member of the turnip virus group, when it occurs alone in cabbage and when it is accompanied by cabbage virus B, a member of the cauliflower virus group. The relation of environmental factors to seasonal fluctuation of symptoms of cabbage mosaic and the interaction of viruses A and B when they occur together in cabbage was also investigated. The black ring virus and the cauliflower mosaic virus were included in one experiment as a further test of their relationship to virus A and virus B, respectively.

MATERIALS AND METHODS

The Jersey Queen variety of cabbage (*Brassica oleracea* var. *capitata* L.) was used throughout the greenhouse experiments. Plants were inoculated in the 3- or 4-leaf stage by methods described previously (*5*). All plants which failed to develop symptoms after a given period were discarded. Plants were grown in 4-inch clay pots in greenhouse soil which was turned and sifted a number of times before being used in order to obtain as great a degree of uniformity as possible. Special effort was made to keep the soil moisture content

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[3] Italic numbers in parentheses refer to Literature Cited, p. 484.

of all pots the same. Uniformity of light conditions was provided by growing the plants directly beneath fluorescent lights in the greenhouse. Temperatures of 16° and 28° C. were maintained in the greenhouse studies, and all plants were kept at a constant temperature of 22° before they were moved to these temperatures for inoculation. In setting up an experiment three times the number of plants needed were potted in order that plants could be closely culled for uniformity at the time of inoculation.

In preparing the inoculum to be tested for virus content [4] small disks of tissue, all of equal area, were cut from the youngest available leaves of the cabbage plants and macerated in a given amount of distilled water until the mixture was completely homogeneous. The usual procedure was to take 1 tissue disk from each of 10 plants and macerate the combined samples in 10 cc. of water. In any comparison an equal number of tissue disks was taken from the same number of plants at each temperature, care being taken that the leaves used were exactly comparable.

The number of local lesions (fig. 1) produced on inoculated leaves of tobacco plants (*Nicotiana tabacum* L. var. Connecticut Havana 38) was used as a measure of the virus concentration in samples of juice extracts. Spencer and Price (*7*) and Price and Spencer (*6*) have pointed out that the local-lesion method merely measures virus activity. Thus only when two virus preparations differ only in the concentration of the infectious entities do local-lesion counts become a measure of differences in virus concentration. It was considered that in these studies the differences in lesion counts at high and low temperatures represented differences in virus concentration since temperature was the only variable involved. The half-leaf method of comparison was used in all experiments carried out under controlled greenhouse conditions. Whole-leaf comparisons were used for inoculations from field-grown plants. Finely powdered carborundum was sprinkled as evenly as possible on both halves of leaves before inoculation. Although virus A will produce a limited number of lesions without carborundum, preliminary trials indicated that the infectivity of the virus was so low that greater variation between halves of the same leaves occurred when carborundum was not used than when it was used. Uniformity tests described in the following section show that the use of carborundum did not introduce any significant variation. All plants were sprinkled with carborundum before any leaf was designated to receive a particular treatment. Any particular treatment was placed an equal number of times on the right and left halves of leaves. Plants used in any one experiment were all of the same age and were carefully chosen for uniformity. Before inoculation the growing point and all excess leaves were removed from the plants. Inocula were applied with glass spatulas.

EXPERIMENTAL RESULTS

INOCULATIONS FROM FIELD PLANTS

Since it had been observed that symptoms of virus A diminished as the fall weather became cooler, it was thought that the concentration of this virus in plants might also diminish as the temperature became lower. Therefore, in the summer of 1941, 27 plants were chosen from

[4] The term "virus content" used in this paper refers only to the virus activity of expressed sap as measured by local-lesion counts on *Nicotiana tabacum*.

FIGURE 1.—Local lesions produced on inoculated leaves of *Nicotiana tabacum* by cabbage virus A. The black-ring virus produces an identical reaction on this host.

a cabbage mosaic field plot as showing typical symptoms of the A+B virus complex. Inoculations to cabbage and tobacco were made on August 23, September 12, October 14, and November 6 from these plants while they were still in the field. During this time the daily mean temperature gradually became lower and the plants withstood a few light freezes. The average mean daily temperature for August, September, October, and November was 22.2°, 18.2°, 11.8°, and 4.2° C., respectively. On November 11 the plants were dug up and placed in pots in a coldframe where the temperature was maintained at about 5°. A fifth inoculation was made on January 29, or some 9 weeks after plants were removed from the field. On that date the plants were transferred to a greenhouse temperature of 24°. A final inoculation was made on February 16. In making the inoculations the youngest leaf tissue available was macerated in a sterile mortar and filtered through several layers of cheesecloth. Equal quantities of the undiluted extract were used to inoculate both cabbage and tobacco plants. Before the plants were mature in the field, inoculum was taken from the inner head leaves. After heads were cut, young leaves from new growth at the leaf scars were used.

In table 1 are given the results of the inoculations from 14 plants. The remaining 13 plants did not survive the long period in the coldframe. The data show an apparent decrease in concentration of virus A as the temperature decreased and an increase in concentration when plants were exposed again to high temperatures. This is indicated by the reaction on both cabbage and tobacco. It is of interest to note that tobacco was infected at lower concentrations than cabbage.

This experiment was repeated in 1942 but unfortunately only three inoculations were made from the plants. A severe freeze in mid-October terminated the experiment. A study of table 2, however, will show that results similar to those of 1941 were obtained.

TABLE 1.—*Concentration of cabbage virus A as measured at frequent intervals in cabbage plants infected with viruses A and B and exposed to decreasing temperatures until January 29 when the temperature was increased to 24° C.; 1941*

Plant No.	Number of lesions on 6 tobacco leaves inoculated [1]—						Type of symptoms produced on cabbage inoculated [2]—					
	Aug. 23	Sept. 12	Oct. 14	Nov. 6	Jan. 29	Feb. 16	Aug. 23	Sept. 12	Oct. 14	Nov. 6	Jan. 29	Feb. 16
1	198	+	0	0	1	68	A+B	B	–	B	A+B	A+B
2	315	+	47	3	0	277	A+B	B	B	B	–	A+B
3	140	+	32	0	0	98	A+B	B	B	B	–	A+B
4	287	+	41	0	2	73	A+B	–	–	B	B	A+B
5	127	+	0	2	1	9	A+B	A+B	B	B	–	A+B
6	162	+	29	2	2	43	A+B	–	B	B	B	A+B
7	211	+	0	5	0	301	A+B	A+B	B	B	B	A+B
8	114	+	19	0	5	81	A+B	–	B	B	B	A+B
9	87	+	21	50	0	34	A+B	B	B	B	B	A+B
10	182	+	0	4	5	57	A+B	B	–	B	B	A+B
11	106	+	42	3	1	16	A+B	–	B	B	B	A+B
12	101	+	0	0	0	36	A+B	–	–	B	B	A+B
13	254	+	139	201	0	68	A+B	A+B	A+B	A+B	B	A+B
14	119	+	17	0	0	49	A+B	B	B	B	B	A+B

[1] + indicates positive reaction; – indicates negative reaction; the number of local lesions were not counted in the inoculation of Sept. 12.

[2] A+B indicates symptoms were those of plants known to contain both virus A and B; B indicates symptoms were those of plants known to contain only virus B; – indicates no symptoms.

TABLE 2.—*Concentration of cabbage virus A as measured at frequent intervals in cabbage plants infected with viruses A and B and exposed to decreasing temperatures; 1942*

Plant No.	Number of lesions on 8 tobacco leaves inoculated—			Symptom type on cabbage inoculated [1]—		
	Aug. 12	Sept. 11	Oct. 10	Aug. 12	Sept. 11	Oct. 10
1	258	8	0	A+B	—	B
2	121	5	0	A+B	B	B
3	88	58	4	A+B	A+B	B
4	112	20	4	A+B	B	B
5	435	118	3	A+B	A+B	B
6	184	161	3	A+B	A+B	B
7	237	109	11	A+B	A+B	B
8	67	38	0	A+B	B	B
9	531	180	0	A+B	A+B	B
10	50	110	0	A+B	A+B	B
11	301	261	4	A+B	A+B	B
12	62	36	0	A+B	B	B
13	34	78	0	A+B	B	B
14	110	11	2	A+B	B	B
15	208	180	0	A+B	A+B	B

[1] A+B indicates symptoms characteristic of plants containing both viruses A and B; B indicates symptoms characteristic of plants containing only virus B; — indicates no symptoms.

In 1942 an experimental planting of kale (*Brassica oleracea* L. var. *acephala* DC.) became heavily infected with cabbage virus A. In late season these plants were removed from the field and placed in a coldframe where the temperature was maintained at about 5° C. On February 3, 1943, after some 3 months at this low temperature, a test inoculation to tobacco was made from 10 such plants, 8 tobacco leaves being used in each test. The kale plants were then transferred to a greenhouse temperature of 24°, and a second inoculation was made on February 23 in exactly the same manner as the earlier inoculation. The results are shown in table 3.

In these experiments involving field plants many uncontrolled variables existed, and if the differences obtained were not so great little significance could be attached to them. In making periodic inoculations from growing plants each inoculum was not exactly comparable to the preceding one. Furthermore, it was necessary to use different tobacco plants for each inoculation, and subsequent data will show that considerable variation exists between tobacco plants in regard to their susceptibility to virus A. In order to circumvent these variables, greenhouse studies were performed in which a similar approach to the problem was made.

TABLE 3.—*Effect of air temperature on the concentration of cabbage virus A in kale plants*

Plant No.	Number of lesions on 8 tobacco leaves—		Plant No.	Number of lesions on 8 tobacco leaves—	
	Feb. 3, 1943 [1]	Feb. 23, 1943 [2]		Feb. 3, 1943 [1]	Feb. 23, 1943 [2]
1	0	282	7	1	879
2	0	497	8	0	382
3	0	0	9	1	640
4	0	414	10	0	519
5	3	1,011			
6	0	364	Total	5	4,988

[1] Inoculation made after plants had grown at 5° C. for 3 months.
[2] Inoculation made 20 days after plants were transferred from 5° to 24°. Four tissue disks were macerated in 4 cc. of distilled water for each inoculation.

GREENHOUSE STUDIES

Uniformity Tests

In making inoculations to tobacco it was necessary to sprinkle powdered carborundum on the leaves in order to get a usable number of lesions, especially if older plants were used. It became necessary then to run uniformity tests in order to ascertain if any significant variation existed between halves of the same leaf when the carborundum method was used. In such trials both halves of all leaves were sprinkled evenly with carborundum, and the same inoculum was applied to all half leaves. Trials were made with both a Latin square and an incomplete block design. The incomplete block design and method of analysis were essentially those described by Youden (*13*) and Youden and Beale (*14*).

In the Latin square design four hypothetical half-leaf comparisons (A, B, C, D) were tested on four plants containing four leaves each according to the following arrangement:

A	B	C	D
B	C	D	A
C	D	A	B
D	A	B	C

In this design each comparison occurred once at each leaf position and once on each plant when the rows are considered as leaves and the columns as plants. This design was especially applicable for virus treatment comparisons, the only requirement being that there be as many leaves on a plant and as many plants as there were treatment comparisons. Unless tobacco plants were grown in large pots the number of available leaves was usually limited to four or five.

By the use of the incomplete block design, uniformity trials were run in which 13 hypothetical half-leaf comparisons (A, B, C, D, E, F, G, H, I, J, K, L, M) were made on 26 plants of 3 leaves each. Each comparison occurred twice at each leaf position and once on the same plant with every other comparison according to the following arrangement:

A	A	B	B	C	C	D	D	E	E	F	F	G	G	H	H	I	I	J	J	K	K	L	L	M	M
B	D	D	E	H	E	F	G	F	L	A	K	J	C	G	B	K	H	A	I	M	L	C	M	I	J
C	E	I	M	D	J	J	M	H	G	G	B	B	I	K	L	E	A	K	L	C	D	F	A	F	H

This design proved especially efficient, since a large number of half-leaf comparisons could be made and at the same time leaf position and plant effects could be measured. It was more applicable than the Latin square in that a much greater number of treatment comparisons could be made. Two such uniformity trials were made. Analysis of variance of the data showed that the variation between halves of the same leaves with the carborundum method fell well within the range of experimental error. No significant difference existed between half-leaf comparisons. A marked gradient of susceptibility existed between leaf positions, the uppermost leaves being least susceptible and the lowest leaves being most susceptible. The middle leaves were intermediate in susceptibility. Although fluctuations occurred, this gradient of susceptibility was consistent and occurred throughout the experiments. A marked variation also occurred between individual plants. Results of uniformity tests in which a Latin square was used were similar to those with the incom-

plete-block design as regards variation between halves of the same leaves, between leaf positions, and between plants. Since these data are being presented elsewhere the detailed lesion counts are not given here.

Temperature and Photoperiod Relations

Since the uniformity trials indicated that the half-leaf method with carborundum would yield reliable comparisons, experiments were performed in which the concentration of virus A was measured in cabbage plants infected with virus A alone and with viruses A and B together and grown under 15-hour (long day) and 6-hour (short day) days at at both 16° and 28° C. Day lengths were controlled by covering the plants with light-proof cages when the respective day length terminated. Air ventilation was provided so that the air temperature inside and outside the cage was the same. Thus with the 6 different treatments 12 half-leaf comparisons were made as follows:

Day length:
- Virus A (long day) at 28° vs. virus A (short day) at 28°.
- Virus A (long day) at 16° vs. virus A (short day) at 16°.
- Virus A+B (long day) at 28° vs. virus A+B (short day) at 28°.
- Virus A+B (long day) at 16° vs. virus A+B (short day) at 16°.

Temperature:
- Virus A (long day) at 28° vs. virus A (long day) at 16°.
- Virus A (short day) at 28° vs. virus A (short day) at 16°.
- Virus A+B (long day) at 28° vs. virus A+B (long day) at 16°.
- Virus A+B (short day) at 28° vs. virus A+B (short day) at 16°.

A vs. A+B:
- Virus A (long day) at 28° vs. virus A+B (long day) at 28°.
- Virus A (short day) at 28° vs. virus A+B (short day) at 28°.
- Virus A (long day) at 16° vs. virus A+B (long day) at 16°.
- Virus A (short day) at 16° vs. virus A+B (short day) at 16°.

One of the treatment comparisons was duplicated each time in order that the same incomplete block design could be used that was used in the uniformity trials With these comparisons the effect of both day length and temperature on the activity of virus A as well as the effect of virus B on the activity of virus A was measured. The day-length effect was studied since it had been observed in nature that the effect of virus A was at a maximum during warm, long days and declined as plants grew into cool, short days. It was not certain whether the reduced activity was due to reduced temperature, to reduced day length, or to a combination of low temperatures and short days It was of interest to measure the effect of virus B on the activity of virus A since it had previously been shown that the effect of these two viruses together was much more severe than that of either virus alone (*5*) Since this increased severity was more pronounced at high temperatures where virus A was apparently more active, it became a question as to whether virus B might not enhance the activity of virus A.

In table 4 are given the results obtained in three separate experiments of this nature In each experiment virus A occurred in much greater amount in cabbage plants grown at 28° C. than in those grown at 16° The F value in each temperature comparison was greater than that at the 1-percent level of probability except in one trial in experiment 1 where it exceeded the 5-percent level of probability.

No significant difference was found between the virus concentrations in plants growing at different day lengths In all long- and short-day comparisons the *F* values obtained were without exception smaller than that at the 5-percent level of probability, indicating that the day lengths studied did not affect the concentration of virus A.

TABLE 4.—*Effect of temperature and length of day on the concentration of cabbage virus A in cabbage plants infected with virus A alone and with viruses A and B together in the greenhouse under controlled conditions*

Half-leaf comparisons with virus—	Temperature (°C.)	Length of day [1]	Experiment 1		Experiment 2		Experiment 3	
			Comparison totals	*F* value [2]	Comparison totals	*F* value [2]	Comparison totals	*F* value [2]
A	28	Long day	[3] 304	0.37	512	0.46	227	0.28
		Short day	342		463		205	
	16	Long	178	.39	142	.76	94	.59
		Short	217		205		126	
A+B	28	Long	352	.00	456	2.84	203	1.11
		Short	353		578		247	
	16	Long	128	.08	179	.11	150	.23
		Short	146		155		170	
A	28	Long	379	10.14**	404	10.26**	321	17.54**
	16		179		172		146	
	28	Short	366	8.12**	500	19.52**	323	17.14**
	16		187		180		150	
A+B	28	Long	272	7.85**	387	13.18**	218	9.53**
	16		96		124		89	
	28	Short	264	6.25*	624	34.59**	318	30.03**
	16		107		198		89	
A	28	Long	435	1.00	421	1.16	279	.55
A+B			372		499		248	
A	28	Short	498	1.79	519	3.63	254	.10
A+B			414		657		267	
A	16	Long	212	.11	211	.25	146	.07
A+B			191		175		135	
A	16	Short	192	.39	198	.10	175	1.11
A+B			153		175		131	

[1] Long day and short day refer to 15-hour and 8-hour day, respectively.
[2] Values given are local-lesion totals in 6 replicates.
[3] **Highly significant; *significant; unstarred values are insignificant.

The concentration of virus A in plants infected with both A and B viruses exactly paralleled that in plants infected with virus A alone. In these comparisons no significant differences were found, either under long or short day, or at high or low temperatures. Furthermore, in the comparisons of virus A concentration at the two temperatures, the differences were similar in plants inoculated with viruses A and B and those with virus A only. This would indicate that virus A is quite independent of virus B within the host plant and that this independence is not altered by such environmental factors as temperature and day length. The increased severity resulting when viruses A and B occur together is apparently due to the additive effect of each virus on the host metabolism and does not result from an enhanced activity of virus A.

Although the length of day used did not affect the concentration of virus A, there was a noticeable effect upon host growth and upon the severity of virus symptoms. Under short day at both high and low temperatures healthy cabbage plants were chlorotic and showed a much shortened growth as compared to plants of long-day exposure. This effect was more pronounced at 16° than at 28°. At both temperatures, symptoms of virus A and of viruses A and B together were less severe and those of virus B more severe under short day than under long-day exposures.

Since it had previously been determined that the incubation period of virus A was much longer at 16° than at 28° C., it was thought that the low concentration of the virus at 16° might be a result of the long period which the virus required to establish itself at 16° rather than a temperature effect after the virus was thoroughly established in the plant.

To test this possibility the following experiment was performed. Virus A and viruses A and B together were each inoculated to 30 young cabbage plants and incubated at a constant temperature of 22° for 12 days. They were then transferred to temperatures of 16° and 28°, half the number being placed at each temperature. Five days after removal to 16° and 28° an inoculation was made to tobacco to test the concentration of virus A. Twenty-five days later a second inoculation was made from the same plants to test the concentration after the lapse of 25 days. The results of the two inoculations are given in table 5. The concentration of virus A in plants at 28° was not significantly greater than the concentration in plants at 16° 5 days after removal from 22°, either in plants infected with virus A alone or in plants infected with both A and B viruses. However, after 30 days' incubation at 28° and 16°, the concentration of virus A in both A and A+B plants was much greater at 28° than at 16°. This would indicate that the long incubation period of virus A at 16° does not account for the low concentration of the virus at that temperature. Even after the virus is thoroughly established in plants, the concentration will rapidly diminish if plants are exposed to a lower temperature for any length of time.

TABLE 5.—*Concentration of cabbage virus A in cabbage plants infected with virus A alone and with viruses A and B together and incubated at 22° C. for 12 days before being placed at temperatures of 16° and 28°*

Half-leaf comparisons with virus—	Temperature	5 days after being placed at temperatures of 16° and 28° [1]		30 days after being placed at temperatures of 16° and 28°	
		Comparison totals [2]	*F* value	Comparison totals	*F* value [3]
A	28°	994	2.45	897	53.80**
	16°	1,100		87	
A+B	28°	753	1.33	1,008	71.96**
	16°	675		72	
A	28°	970	.37	887	1.50
A+B		1,011		1,022	
A	16°	921	2.27	106	.079
A+B		819		75	

[1] In the first inoculation a randomized block design was used; each comparison was made on 2 plants of 3 leaves each. In the second inoculation a Latin square design was used; each comparison was made once on 12 different plants.
[2] Values given are local-lesion counts.
[3] **Highly significant.

One experiment was performed to determine whether viruses A and B would become established in plants at temperatures much lower than 16° C. Fifteen cabbage plants were inoculated with each of virus A and viruses A and B together and placed immediately in a coldframe where the temperature was held at about 5°. At the same time an equal number of plants was inoculated with each virus or viruses and placed at 28°. After 60 days' incubation the plants were removed from the coldframe to the 28° temperature, and an inocula-

tion was made from both groups of plants to test the concentration of virus A on the day of removal from the coldframe. During this 60-day interval the plants in the coldframe had made very little growth (two or three small leaves above inoculated leaves), and except for one inoculated leaf, they showed no evidence whatever of virus infection. However, after 48 hours at 28° systemic symptoms developed very rapidly. The A-infected plants developed symptoms typical of the virus A, and the A+B plants developed typical A+B symptoms. After a 30-day interval at 28° a second inoculation was made from both groups of plants in a manner similar to the first. The results of both inoculations are given in table 6.

TABLE 6.—*Concentration of cabbage virus A in cabbage plants infected with virus A alone and with viruses A and B together and incubated at 5° and 28° C. for 60 days and later at 28° for 30 days* [1]

Half-leaf comparisons with virus—	Temperature	After 60 days' incubation at temperatures 28° and 5°		30 days after removal of plants to 28° and 5°	
		Comparison totals [2]	*F* value [2]	Comparison totals	*F* value
A	28°	385	22.07**	309	1.38
	5°	3		263	
A+B	28°	609	56.10**	359	.11
	5°	0		372	
A	28°	424	.62	250	.48
A+B		488		277	
A	5°	3	.001	191	.71
A+B		1		224	

[1] In both inoculations a Latin square design was used; each comparison occurred once on 8 different plants.
[2] Values given are local-lesion counts.
[3] ** Highly significant.

It is noted that even after 60 days' incubation at 5° C. the concentration of virus A in systematically infected leaves was almost nil. However, when the same plants were exposed to a high temperature for 30 days, the virus concentration reached a level which was not significantly lower than that in plants which had grown the full 90 days at 28°. Apparently the virus was produced very slowly at this low temperature, but the rate increased rapidly when the plants were exposed to a higher temperature. Much of this condition was no doubt due to the reduce activity of the host. The very small amount of growth produced would indicate that the rate of host metabolism was very low.

In a previous paper (*5*) it was shown that the symptom severity of the black ring virus in relation to temperature was quite similar to that of virus A, while that of virus B was quite similar to that of the cauliflower mosaic virus. In the same report strain relationships in these two groups of viruses were established. Although both virus A and the black ring virus were favored by high temperatures, virus A was distinctly more severe than the black ring virus at high temperatures, whereas at low temperatures the reverse was true. The concentration of these two viruses at high and low temperatures was measured in plants infected with the viruses alone and in combinations with virus B and cauliflower mosaic virus, to ascertain if this measur-

able difference in symptom reaction was due to a reduced activity of the black ring virus at 28° and an increased activity at 16°. The cauliflower virus was included to see if it would react like virus B when in combination with virus A or the black ring virus.

The results of this experiment (table 7) show that the concentration of the black ring virus closely paralleled that of virus A, since both viruses occurred in significantly greater amounts at 28° than at 16°, when alone and when in combinations with virus B or the cauliflower mosaic virus. This indicates, as suggested in an earlier paper (*5*), that the effect of temperature upon any host-virus reaction depends upon the specific host-virus complex and may be due to the temperature effect upon the host, virus, or both. Although no half-leaf comparisons were made between virus A and the black ring virus, the results do not indicate that either occurs in significantly greater amounts than the other.

TABLE 7.—*Effect of temperature on the concentration of cabbage virus A and cabbage black ring virus in cabbage plants alone and in combination with virus B and cauliflower mosaic virus in the greenhouse*

Half-leaf comparisons with virus—	Temperature	Comparison totals [1]	*F* value [2]
A	28°	625	65.91**
	16°	209	
A+B	28°	507	59.13**
	16°	113	
A+cauliflower	28°	542	66.55**
	16°	124	
Black ring	28°	498	51.58**
	16°	130	
Black ring + B	28°	485	38.76**
	16°	166	
Black ring+cauliflower	28°	514	48.82**
	16°	156	

[1] Values given are lesion totals of 6 replicates in a randomized block.
[2] **Highly significant.

Virus B produces prominent and enduring symptoms at 16° C. and mild symptoms which are readily masked at 28°. To determine whether the concentration of this virus was affected by temperature, inoculum was taken from plants growing at each temperature and used to inoculate 100-plant lots of cabbage. An equal amount of comparable leaf tissue was used for each inoculation. When the virus extract was diluted 1:1, little difference was found in the number of plants infected. Since virus B has a very low tolerance to dilution, it was thought that by diluting the virus extract any difference in concentration at 28° and 16° could be more readily detected. Table 8 shows that on the basis of inoculations with extract diluted 1:50 the concentration was 100 percent greater at 16° than at 28°. Although this method of measuring virus concentration is probably less accurate than that used for virus A, it was the only method available since virus B is not known to produce local symptoms on any host. If virus B does occur in greater amount at low temperatures, as indicated in this one test, it would seem that the prominence of symptoms at low temperatures is related to the concentration of the virus. This condition has its counterpart in the reaction of virus A and black ring virus to high temperatures.

TABLE 8.—*Effect of air temperature on the concentration of cabbage virus B in cabbage plants*

Source of inoculum	Number of plants infected out of 100 inoculated	
	Extract diluted 1:1	Extract diluted 1:50
Cabbage at 28° C.	94	45
Cabbage at 16° C.	90	91

In all the preceding experiments virus A was found to occur in much greater concentration in cabbage plants growing at 28° C. than in plants growing at 16°. Since the rate of transpiration is probably greater at 28° than at 16°, a test was made to see whether the moisture content of the leaf tissue of plants at 16° was greater than that of plants at 28°, and whether in consequence the virus would be diluted upon maceration more at 16° than at 28°. Disks of tissue were cut from leaves at both temperatures and weighed immediately. These disks were taken at the same time and from opposite halves of the same leaves as the disks taken for the concentration tests. The results, given in table 9, show that there was little difference in the moisture content of leaves grown at the two temperatures.

TABLE 9.—*Moisture content of healthy and virus-infected cabbage leaves growing at 16° and 28° C.*

Plant infected with—	16° C.			28° C.		
	Fresh weight (gm.)	Dry weight (gm.)	Percent	Fresh weight (gm.)	Dry weight (gm.)	Percent
Virus A	[1] 1.115	0.117	89.50	1.085	0.112	89.70
Viruses A+B	1.113	.100	91.00	1.160	.100	91.30
Healthy	1.210	.146	87.90	1.070	.112	89.50
Total	3.438	.363	89.40	3.315	.324	90.20

[1] Weights given are for 10 tissue disks, 1 from each of 10 plants.

DISCUSSION

The concentration of virus A has been shown to decline as the symptoms of the disease recede with the gradual lowering of temperature in the late summer and autumn in the latitude of Wisconsin. Likewise limited evidence is presented that the concentration of virus B increased as the weather became cooler. The black ring virus showed the same temperature-concentration effect as virus A, to which it is closely related. Length of day had no measurable effect upon concentration of the viruses, but the symptoms of virus A alone and viruses A and B together were less severe, and those of virus B alone more severe, under short- than under long-day exposures. It is of particular interest to note that with virus A and with virus B there is a parallel increase of symptoms with increase in virus concentration.

Moreover, these two unrelated viruses appeared to increase or decrease in the same host in response to temperature without relation to each other. This, together with cross-immunity results presented in another paper (*5*), suggest the possibility that these unrelated viruses do not compete with each other in the process of conversion of normal proteins to the respective virus proteins after infection takes place. The cauliflower mosaic virus assumed the same role as virus B in combination with virus A. The black ring virus assumed the same role as virus A in combination with either the cauliflower mosaic virus or virus B.

There is nothing in the relation of virus concentration and temperature to explain the fact that at moderate temperatures (20° C.) the black ring virus causes greater necrosis on cabbage than virus A while at high temperature (28° C.) virus A causes greater chlorosis and mottle than the black ring virus.

It is not unlikely that many viruses vary in concentration with the temperature at which the host is grown. In fact, the temperature-concentration relation may be found to vary for the same virus in different hosts. The profound effect of temperature on virus concentration noted with the viruses studied in this investigation has led the writers to emphasize the importance of considering the temperature at which the host is grown when a given virus is being examined as to physical properties. This is particularly true since the original concentration of the virus extract may have a considerable influence on the point at which inactivation is noted as a result of dilution, aging, or exposure to given temperatures.

SUMMARY

The results obtained in this study are explanatory of observations on the epidemiology of the cabbage mosaic disease as it occurs in the Midwestern States. Symptoms of virus A appear in early summer when temperatures are high and remain severe until the cooler fall temperatures appear. The A symptoms then recede and symptoms of virus B attain prominence. Inoculations from field plants in late season yield virus A only in small amounts, and very often not at all, but they yield virus B in abundance. These conditions are no doubt due to a reduction in the rate of production of virus A and perhaps to an increase of virus B at low temperatures. Moreover, in the southern belt of States where cabbage is grown in winter and early spring virus B is more prevalent than virus A. In California Tompkins et al. (*10*) states that the black ring disease of cabbage occurs in the winter and is uncommon during the summer months. Although in these studies the effect of temperature on concentration of the black ring virus was found to be affected by temperature similar to that on virus A, it was earlier shown that the black ring virus produced more severe necrosis at low temperatures than did virus A. However, the failure of the black ring disease to occur in the summer months cannot be explained by the results obtained in these temperature studies.

Quantitative studies have been made to determine the effect of air temperature on the concentration of cabbage virus A, cabbage black ring virus, and cabbage virus B in cabbage plants. The effect of day

length on the concentration of cabbage virus A was also studied. Results obtained were subjected to statistical treatment.

Cabbage virus A and cabbage black ring virus were shown to occur in significantly greater concentration in cabbage plants growing at 28° C. than in cabbage plants growing at 16°, both when occurring in cabbage alone and together with either cabbage virus B or cauliflower mosaic virus.

The concentration of cabbage virus A in cabbage growing in a 15-hour day at either 28° or 16° C. was not significantly different from that in plants growing in an 8-hour day. This was true whether the virus occurred alone or together with virus B in the host.

When plants infected with virus A and viruses A and B together were incubated at 22° C. and later moved to houses of 16° and 28°, the concentration of virus A in plants at 16° fell significantly below the concentration in plants at 28°.

When plants were inoculated and kept at 5° C. for 60 days, the systemic build-up of virus A was practically nil. When the plants were moved from the 5° temperature to a temperature of 28°, the concentration of virus A after 30 days increased until it was not significantly different from that in plants which had grown the full 90 days at 28°.

No significant difference was found between the concentration of virus A in plants infected with virus A alone and the concentration in plants infected with both viruses A and B. It is suggested that the increased severity of symptoms of viruses A and B together over that of either virus alone results from the additive effect of each virus on the metabolism of the host.

Results obtained indicate that virus B occurs in greater amounts in plants grown at 16° C. than in plants grown at 28°, although the method used was less accurate than that employed in measuring the concentration of virus A.

The concentration of virus A in field plants progressively declines as plants grow into increasingly cool temperatures in the fall.

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PHYSIOLOGICAL INTERNAL NECROSIS OF POTATO TUBERS IN WISCONSIN[1]

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INTRODUCTION

In the sandy, gravelly soils of Adams, Juneau, Portage, Waupaca, Waushara, and Wood Counties of central Wisconsin (fig. 1), and to some extent in Barron and Washburn Counties in the northwest, and in Washington County in the southeast, serious losses to the late

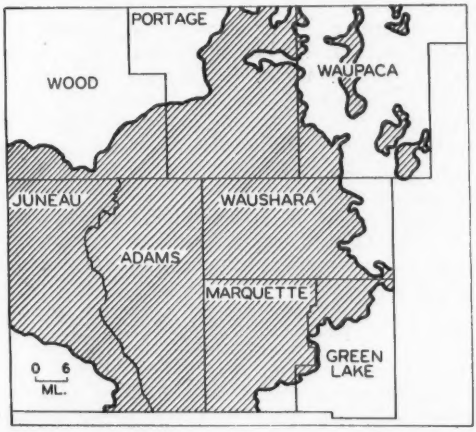

FIGURE. 1.—Sandy soil area of central Wisconsin shown by cross hatching.

commercial potato crop are sustained during certain seasons because of internal necrosis (physiological brown or rust spot) of the tubers.[3] This internal tuber disease condition greatly reduces the culinary, dehydration, and chip quality because the necrotic lesions remain as discolored, fibrouslike masses in the final product. The malady is most prevalent during seasons in which temperatures are above normal and precipitation below normal. Internal brown or rust spot was very prevalent in the State in 1937, 1939, and 1943, more prominent than usual in 1938 and 1942, and moderate in 1940 and 1941.

The present paper is a report of field and laboratory investigations which have been under way since 1938. Several preliminary reports of the work have been made (*1, 2*).[4] Laboratory studies were con-

[1] Received for publication June 14, 1944.
[2] The writers are indebted to Clifford J. Kinschi for valuable assistance in field operations.
[3] The type of internal tuber necrosis under discussion is of the blotch or spot type, not to be confused with the arclike lesion "spraing" type of internal necrosis.
[4] Italic numbers in parentheses refer to Literature Cited, p. 503.

ducted at Madison, Wis., and the field studies were carried out on Plainfield sand in the commercial potato area at Hancock, Waushara County, Wis. The occurrence of internal tuber necrosis in Wisconsin prior to the present investigation has been reported (*12*, *13*).

METHODS AND MATERIALS

RECORDING INDEX VALUES

The method of calculating amount and degree of internal tube necrosis was similar to that used by the writers (*24*) in their analysis of scab resistance in potatoes. The tubers for examination (random sample of 100 or more tubers per replicate) were cut to expose as much of the internal necrotic tissues as possible, necessitating in some cases the cutting of tubers two or three times. Severity of necrosis was recorded in four arbitrary classes as follows: (1) Clean—no macroscopic necrosis; (2) slight—scattered flecks; (3) moderate—diffused irregular flecked areas; (4) severe—diffused and irregular large concentrated lesions. Tubers representative of classes 2, 3, and 4 are shown in figure 2. The index number 0 indicates all tubers free from internal necrosis, while 100 represents a case in which all tubers had severe internal necrosis. To reduce the error in recording class divisions, all classifications were recorded by the senior author. The Katahdin variety was used throughout all laboratory and field experiments, except varietal resistance tests, because of its susceptibility to internal tuber necrosis and its adaptability to growing conditions in central Wisconsin.

HISTOLOGICAL METHODS

Material for histological study was collected directly from a field planting of Katahdin potatoes at Hancock, Wis. All tissues selected were fixed immediately after they were cut by placing them directly in a solution consisting of 10 cc. of commercial 40-percent formalin, 5 cc. of glacial acetic acid, and 85 cc. of 50-percent ethyl alcohol. Tissues were evacuated under slight suction while in the fixative. The normal butyl alcohol schedule (*27*) was followed for dehydration. The tissues were embedded in tissue-mat paraffin with a melting point at 52°–54° C. Sections were cut 10 microns in thickness and mounted serially. The stain combination of safranine (1 percent in 95-percent ethyl alcohol) followed by fast green (saturated solution in absolute ethyl alcohol) was found to be most satisfactory for tissue differentiation.

SYMPTOMS

Internal necrosis is entirely a malady of the growing tubers; there are no manifestations of the disorder in aerial parts of the plant in the field during the growing season. Affected tubers show no external symptoms. The necrotic lesions appear translucent when thin tuber sections are exposed to transmitted light. Thicker tissue sections show the flesh to be marked wtih sparse amber-colored to reddish-brown specks converging into darker flecks or compact dark-brown irregular lesions scattered indiscriminately throughout the pith within the vascular cylinder (fig. 3, *A*, *B*). Less frequently they radiate from the pith toward the vascular ring (fig. 3, *C*) or appear to follow

the lines of the internal phloem. In some cases affected tissues are confined to a necrotic mass (fig. 3, *D*), but as a rule they vary in size from mere specks to irregular blotchlike areas having a diameter of one-fourth to one-half inch. Smaller lesions often coalesce to produce large irregular necrotic areas.

In cut tubers exposed to the air the internal necrotic tissues change little in color, although there is a slight increase in intensity of the rusty color already present. The affected areas are firm, corky, and

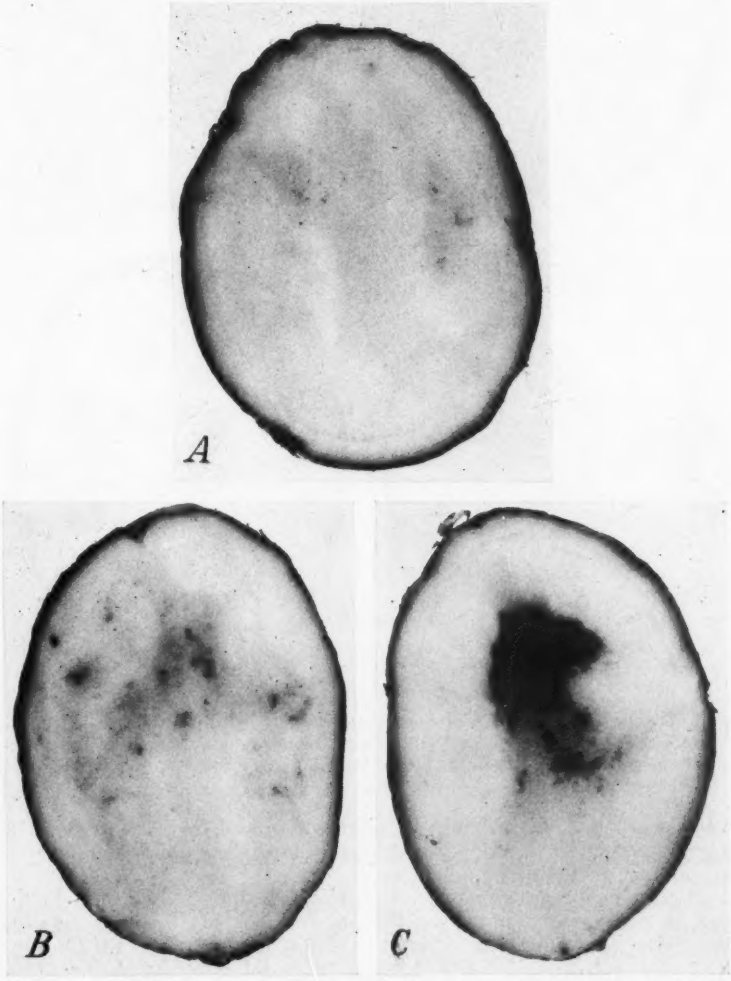

FIGURE 2—Index classes. Sectioned tubers showing representatives of three classes of internal necrosis used in determining the disease index. *A*, slight; *B*, moderate; and *C*, severe.

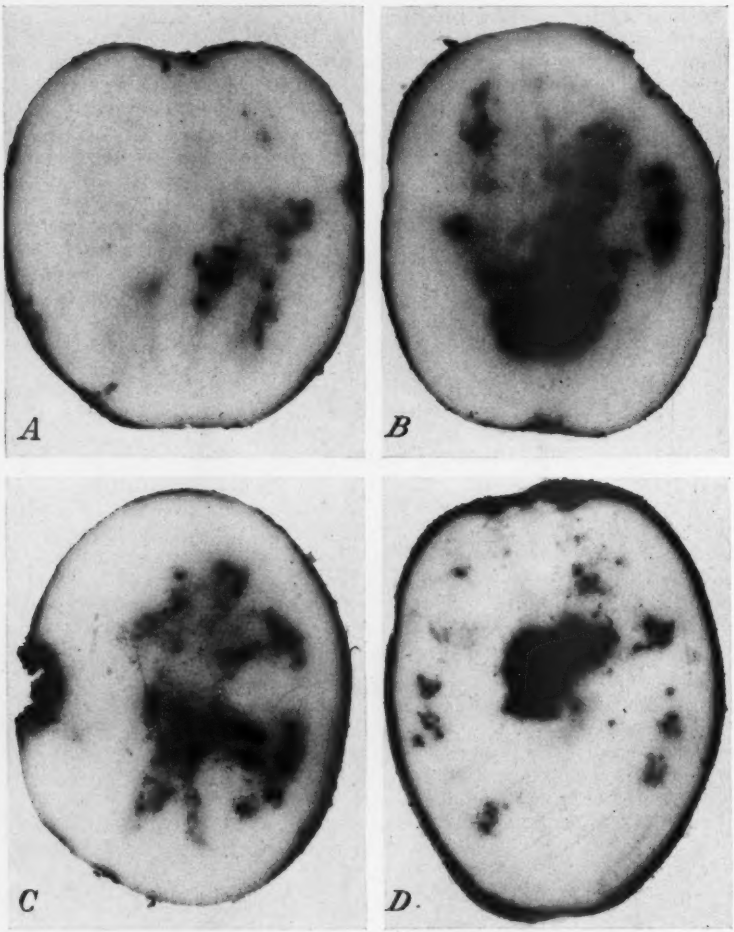

FIGURE 3.—Tubers of the Katahdin variety cut longitudinally, showing types of internal lesions. *A* to *D*, Necrotic areas occurring indiscriminately throughout the pith tissues within the vascular cylinder. Photographs taken at time of harvest.

leathery in texture. Tissue break-down in the form of cavities or internal tuber decay following internal necrosis has not been observed in the field or in storage.

The internal necrosis under discussion is not reticulate and should not be confused with net or phloem necrosis of the potato tuber associated in certain potato varieties (Spaulding Rose, White Hebron, Irish Cobbler, and Green Mountain) with infection by the leaf roll virus (*10*); nor should it be confused with frost necrosis of the lesion type (*13*, *26*) or internal mahogany browning that occurs in the

Chippewa, Katahdin, and Cobbler varieties following long exposure to medium low temperature (*11*). Internal necrosis occurs, however, in the same areas in central Wisconsin as does the potato yellow dwarf disease. The tuber necrosis produced by the yellow dwarf virus is similar in many respects, both macroscopic and microscopic, to the nonparasitic internal necrosis considered in this paper (*14*, *25*).

When tubers affected with internal necrosis are boiled or baked, the necrotic lesions remain as hard, dark-brown, corky masses in the softened pith tissues and the culinary quality is very greatly reduced. The type of internal discoloration referred to as internal necrosis is entirely distinct from the darkening or blackening of potatoes which sometimes occurs after boiling (*23*). The lack of distinguishable external tuber symptoms of this malady also increases greatly the cost in potato dehydration and chip manufacture because the internal disorganization is not ordinarily detected until the cutting stage.

The disease described above has much in common with types of nonparasitic internal necrosis of potato tubers reported from many parts of the world (*3*, *5*, *6*, *8*, *9*, *16*, *17*, *19*, *20*, *21*); in fact, it may be identical with some of the cases noted. There has been no attempt in this study, however, to identify the Wisconsin malady with any described elsewhere. In certain of the previously described occurrences a correlation of the disease with high temperature and low moisture, as found in Wisconsin, has been recorded (*4*, *5*, *6*, *15*, *17*, *19*, *22*).

PATHOLOGICAL HISTOLOGY

Microscopical studies of affected areas of the tuber indicated distinct phases in the development and increase of the necrotic tissues in the internal and external medulla. The early stage of necrotic development is marked in apparently normal parenchyma by a slight darkening of the cell walls, which usually spreads along the walls from the corners of the cell. The abnormal discoloration was seen to affect two or more corners of the cell. The intercellular substance (middle lamella) in the region of the slightly thickened cell walls is also somewhat darkened (fig. 4, *A*). The second stage is characterized by the aggregation of granulate protoplasm against the cell walls and intensified thickening and discoloration of portions of the walls. This is accompanied by darkening of the walls of surrounding parenchymatous cells (fig. 4, *B*). In the third stage the affected cells tend to aggregate and collapse as a result of pressure exerted by abnormal division of contiguous cells. In addition, abnormal cell groups arise in the area of dividing cells, separated from similar affected groups, and become incorporated in the mass of dividing cells (fig. 5, *A*). In the final stage of tissue break-down there is a concentration of collapsed necrotic cells resulting from the increased pressure brought about by division of surrounding cells. The affected areas or cell groups are cut off or isolated from normal parenchyma cells by a cork meristem in contiguous parenchymatous tissue which subsequently forms a barrier between the normal and necrotic area and prevents further development of necrotic cells (fig. 5, *B*). There are no lysigenous cavities formed by disruption or disintegration of tissues in the internal necrotic pith areas or in the lateral branch medullary parenchyma. These observations of necrotic tissue development are somewhat similar to those reported for internal rust spot (*17*).

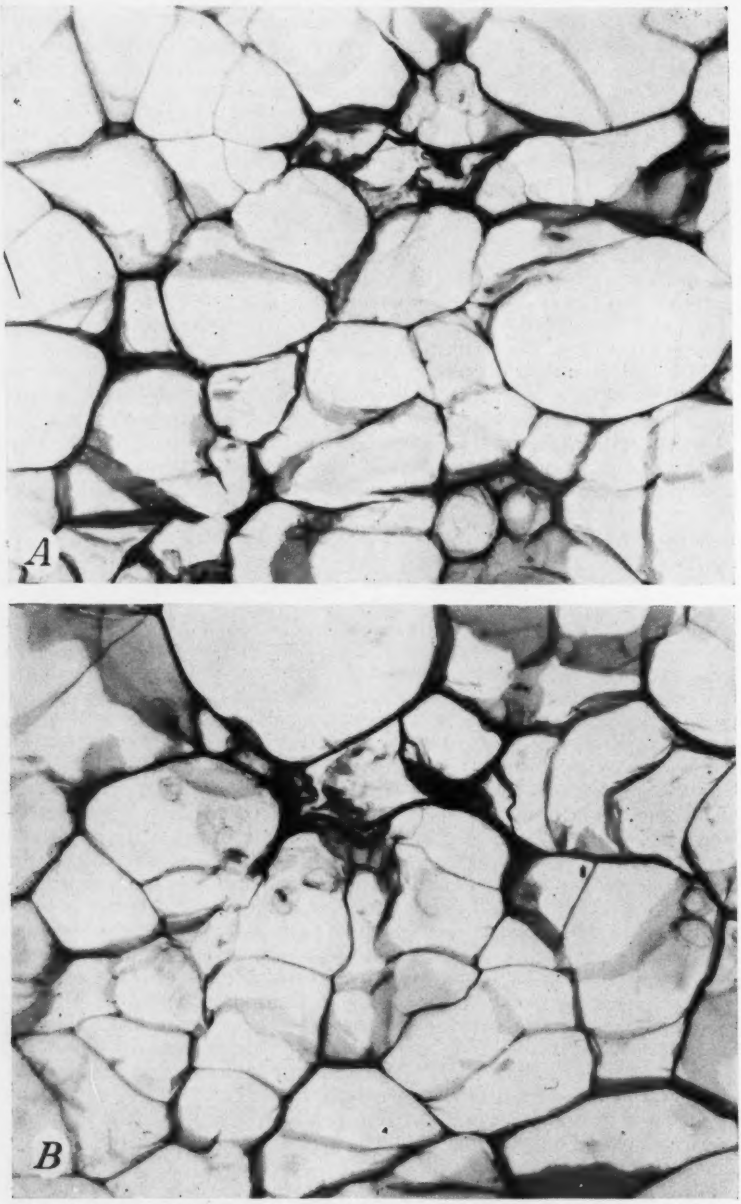

FIGURE 4.—Photomicrographs of tuber sections showing early stages in the formation of internal lesions: *A*, Early darkening of cell protoplast and thickening and darkening of cell walls; *B*, intensified cell-wall thickening and discoloration. X400.

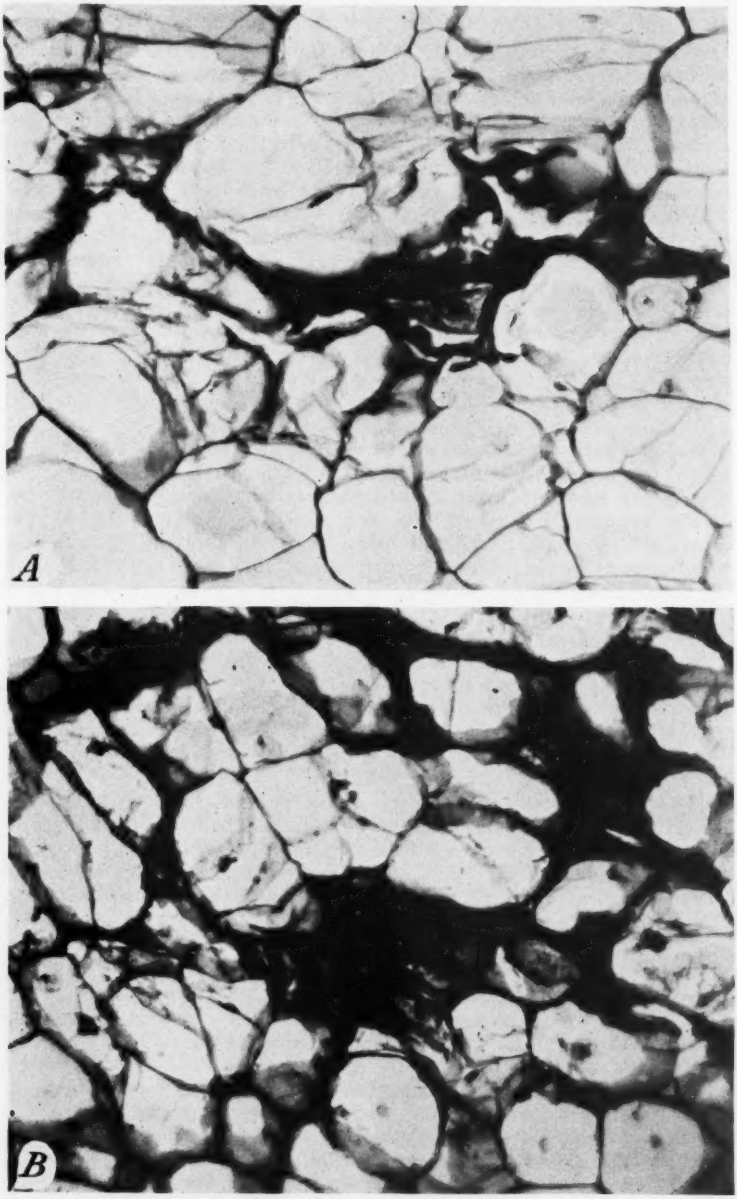

FIGURE 5.—Photomicrographs of tuber sections showing advanced stages in the formation of internal lesions: *A*, Aggregation of collapsed cells and increased division of surrounding parenchyma cells; *B*, increase of necrotic and collapsed cells to form lesions. X400.

MICROCHEMICAL REACTION OF NECROTIC TISSUE

Mircochemical studies of affected and adjacent tissue were made on sections of freshly cut tubers, sectioned either by the freehand method or on the freezing microtome. Reactions were studied in both early- and late-harvested tubers. A considerable amount of suberin or suberized deposits was found in the cell walls of the freshly cut, discolored tissues. Walls of the disorganized cells of the lesions reacted to the suberin test in all cases where this type of tissue had been formed. The thickened walls of the disorganized peridermlike cells toward the periphery of the lesions also showed suberization. These outer cells became suberized, no doubt, as a reaction against the necrotic cell development in the early stages of disorganization and later, as the area increased, became incorporated in the corky meristem tissue. The greatest amount of suberized tissue was found in the cell walls contiguous to necrotic tissues. The presence of pentoses in the center portion of necrotic areas was shown, indicating that they accumulated in walls of the collapsed cells as they became thickened. No definite indication of formation of lignin was found.

Walls of cells in the compact necrotic areas and in the surrounding cells of the corky meristem did not give a positive test for cellulose. If cellulose does remain in the cell walls as they break down, its presence is apparently masked to a large extent by the accumulation of other substances. No positive reaction in tests for tanning or callose were secured in the dense necrotic areas; however, slight reactions indicated their presence in the outer cells of the lesions. A marked and consistent tendency for the thickened cell walls in and around the disintegrated area to show the presence of pectic substances was found.

Freshly cut necrotic areas showed the presence of proteins, dense cytoplasm, and anomalous crystals adjacent to the internal lesions. Where the lesions represented advanced stages and cells had collapsed and lost their identity, crystals were numerous. Such crystals were also found, but to lesser extent, in the outer cortical cells of affected tubers.

In cells composing the affected areas, starch grains were lacking, indicating a disturbance of metabolic processes. Starch grains occurred normally, however, in the adjoining parenchyma.

Necrotic lesions and contiguous parenchyma tissues gave a negative test for solanine, although tissues 1 to 2 cm. from the necrotic areas gave a positive test.

FIELD STUDIES

SEASONAL DEVELOPMENT OF INTERNAL NECROSIS

To secure adequate data as to seasonal development of internal necrotic lesions during tuber growth, the following field-plot experiments were carried out during the growing seasons of 1939 and 1940. A planting of 700 hills of the Katahdin variety was made on Plainfield sand at Hancock and received normal cultivation and frequent spraying with 5–5–50 bordeaux mixture and calcium arsenate. Tubers were harvested from 25 hills of each of 4 randomized replicates at weekly intervals during the season, beginning 73 days after planting. The necrotic index for each sample at each harvest date was determined as soon after collection as possible.

Comparative indices for the two seasons are given in table 1. There was a considerable increase in amount and severity of necrosis in the tubers as the season advanced. The difference in disease index between early- and late-harvest tubers for both seasons is quite marked. In general, the small-sized tubers of 1⅞-inch grade and under showed considerably less necrosis than tubers of U. S. No. 1 size. This is in agreement with observations of tuber size in relation to types of internal physiological tuber necrosis reported by others (*4, 15*). The correlation between tuber size and intensity and degree of necrosis indicates a relationship between growth processes and development of necrosis.

TABLE 1.—*Seasonal increase of internal tuber necrosis*

Number of days after planting	Disease index—		Mean index	Number of days after planting	Disease index—		Mean index
	1939	1940			1939	1940	
73	19	12	15.5	101	30	27	28.5
80	21	17	19.0	108	34	32	33.0
87	25	21	23.0	115	37	35	36.0
94	26	23	24.0	122	42	37	39.5

There was no measurable increase in the amount or severity of internal necrosis when tubers were cut and examined after a period of 8 months in storage at 40° F., following all harvests for the years 1939, 1940, 1941, and 1942. The data thus secured are further substantiated by data collected during the early and late part of the storage period in commercial warehouses and cellars in central Wisconsin during these years.

Moderately affected Katahdin tubers from the 1937 Plainfield sand plot planted at the Coddington station on muck soil for two successive seasons, 1938 and 1939, produced healthy, vigorous foliage and tubers free from internal necrosis and of excellent culinary quality. When the 1939 stock was again planted on Plainfield sand at Hancock in 1940 the tubers in the resulting crop showed an internal necrosis index of 37.5. Plantings of moderately affected seed at Madison in the field and greenhouse for five successive seasons, 1939 to 1943, inclusive, also yielded tubers free from internal necrosis. Similar examples of potato seed affected with certain types of internal physiological necrosis yielding normal tubers on being planted to muck or heavy soils have been reported (*3, 8, 16, 21*).

DEPTH OF TUBER IN THE SOIL IN RELATION TO INTERNAL NECROSIS

In a preliminary trial to ascertain differences in the occurrence and development of internal necrosis in relation to tuber depth in the soil, random samples of the Katahdin variety were taken from a commercial field planting shortly before harvest in 1937. Three groups of 100 tubers each were selected: (1) Tubers with not over 1 inch of soil cover; (2) tubers with 1 to 2 inches of soil cover; and (3) tubers with over 2 inches of soil cover. Sectioned tubers of the three groups showed great differences in both type and severity of internal necrosis. Tubers in groups 2 and 3 exhibited only diffuse internal necrosis of a type shown in figure 2, *B*, whereas group 1 showed a high percentage

of irregular concentrated lesions of a type shown in figure 2, *C*. The indices varied from 0.33 in tubers developing with over 2 inches of soil cover to 37.33 in those with a portion of the tuber exposed to 1 inch of soil cover.

In field trials conducted during 1938 to 1941, inclusive, the differences in severity and type of internal necrotic lesions between tubers developing with 1 inch or less of soil cover and those with more than 1 inch of cover were again quite consistent. Very few tubers showing irregular concentrated lesions were found in the two groups with soil cover over 1 inch, while, as recorded in table 2, there was a consider-

TABLE 2.—*Tuber depth in relation to internal necrosis*

Tuber sample	Disease index—					Mean index
	1937 [1]	1938 [2]	1939 [2]	1940 [2]	1941 [2]	
Group 1—exposed to 1 inch of soil cover	37.33	41.66	47.22	39.10	38.33	40.72
Group 2—1 inch to 2 inches of soil cover	13.33	16.66	18.30	13.28	14.29	15.17
Group 3—over 2 inches of soil cover	.33	.54	.71	.43	.47	.49

[1] 100-tuber samples at each depth.
[2] Mean of 4 replicates—100-tuber samples at each depth.

able decrease in the incidence of internal necrosis in tubers developing with over 2 inches of soil cover. In a series taken from a commercial planting in 1941, tubers from late-planted Chippewa and Russet Rural varieties showed the same relative differences in the incidence of internal necrosis in relation to the depth of soil cover during growth.

Internal tuber necrosis has not been observed in any variety under test planted as an early (late April or early May) crop on Plainfield sand. The late-maturing varieties, Chippewa, Katahdin, and Sebago now being planted early on a commercial scale in central Wisconsin, usually produce table stock of excellent quality with no internal tuber necrosis.

RELATION OF VARIETY TO INCIDENCE OF INTERNAL NECROSIS

A field study of varietal differences in incidence of internal necrosis was initiated in 1938 at Hancock on Plainfield sand. Trials were continued at this location during 1938 to 1943, inclusive. Early, midseason, and late-maturing standard varieties and recently introduced varieties were included. All plantings were made at the usual planting time (early June) for the late commercial crop in this area and received normal cultivation and spraying (5–5–50 bordeaux mixture and calcium arsenate) throughout each season. Indices of internal necrosis were determined each season shortly after the harvest of the crop in four replicates for each variety. The data and a tentative classification of varieties and strains are presented in table 3.

Significant measurable differences in the incidence of internal necrosis are to be noted when the 22 varieties under test are compared. Triumph, a standard early variety in the northern potato-producing areas of Wisconsin (Oneida and Vilas Counties) but not grown as an early variety in the central area, except under irrigation, has consistently shown no internal necrotic discoloration. Houma, a recently introduced variety, has shown a very low incidence in each

of the 3 years under test, exhibiting when affected only a slight diffused type of internal necrosis (fig. 2*B*). Rural New Yorker, Russet Rural, and Katahdin, on the other hand, have been among the most susceptible. The newer varietal introductions under test, Pontiac and Red Warba, were affected very much less by internal necrosis than Sebago, Earlaine, Mesaba, or Sequoia. It is important to note that Chippewa was distinctly less susceptible than Katahdin.

White-blossomed Cobbler, Russet Burbank, Spaulding Rose, and White Rose showed intermediate degrees of disease incidence. Green Mountain, Hundred Day Cobbler, and Columbia Russet are about as susceptible as Katahdin. Harmony Beauty was outstanding in its susceptibility to internal necrosis, exhibiting a severe irregular blotching necrosis (class 4, fig. 2, *C*).

TABLE 3.—*Reaction of potato varieties to internal necrosis*

Variety	Disease index—						Mean index	Number of years under test
	1938	1939	1940	1941	1942	1943		
Triumph	0	0	0	0	0	0	0.0	6
Houma	1	2	2				1.7	3
Pontiac			6	3	3	5	4.4	4
Red Warba		14	12	8	5	7	9.2	5
White-blossomed Cobbler [1]	11	10					10.5	2
Russet Burbank	18	10	14				14.0	3
Spaulding Rose		12	14	17			14.3	3
White Rose		18	10	15	19		15.5	4
Chippewa	20	14	14	27	22	19	19.3	6
Sebago			16	20	21	22	19.8	4
Irish Cobbler	23	22	18	31		19	22.6	5
Earlaine	27	22	16	28	24		23.4	5
Sequoia				30	32	29	30.3	3
Mesaba	36	30	26				30.6	3
Green Mountain	28	40	26	31			31.2	4
Hundred Day Cobbler	35	38					36.5	2
Columbia Russet	38	38					38.0	2
Rural New Yorker	38	32	31	56			39.2	4
Katahdin	41	46	34	30	39	47	39.5	6
Russet Rural	41	40	32	39	48	43	40.5	6
Pioneer Rural [2]	49	54					51.5	2
Harmony Beauty					52	57	54.5	2

[1] Natural seedling of Cobbler.
[2] Smooth mutant of Russet Rural.

It should be pointed out that the Sebago variety has distinct advantages as a late potato for the large commercial area of central Wisconsin, because of its intermediate resistance to internal necrosis, tolerance to late blight in both foliage and tuber, and high field resistance to the yellow dwarf virus (*14*).

Differences in varietal susceptibility to physiological internal necrosis have been reported for British, Dutch, and German varieties of potatoes (*4, 5, 7, 15, 18, 19*). In addition to 10 Dutch and 3 British potato varieties listed as resistant to a type of internal necrosis occurring in Holland, 3 American varieties, Burbank, Irish Cobbler, and Triumph were included (*19*).

SOIL MANAGEMENT AND THE INCIDENCE OF INTERNAL NECROSIS

Soil Mulching

In a study of the effect of soil mulch on the incidence of internal tuber necrosis, a series of field plots was laid out in randomized repli-

cated order in 1938 on Plainfield sand at Hancock, Wis. The following treatments were used: (1) Addition of 2 inches of rye straw; (2) 1 inch of muck soil cover; (3) shallow soil mulch provided by frequent cultivation; (4) no mulch; (5) soybean shade. The purpose of these soil-mulch treatments was to create, if possible, soil temperature differences around the growing tubers. In another treatment fresh-cut alfalfa was incorporated as green manure in the row before planting.

All mulch plots were planted and cultivated during the early stage of growth according to the current farm practices in the Hancock area. After the plants reached the early branching stage just prior to tuberization, the entire planting was weeded by hand before the application of the mulch treatments. The plots sown early to soybeans were weeded by hand during the entire season. Potato insects and foliage diseases were kept under control throughout the growing season by a general spray program.

The incidence of internal necrosis in the various treatments is shown in table 4. The most significant difference is to be noted when the straw mulch is compared with each of the other types. Incorporation of fresh-cut alfalfa as green manure and muck-soil mulch resulted in a slightly higher necrotic index than any of the other treatments.

TABLE 4.—*Internal necrosis index on soil management plots*

Type of soil management	Disease index [1]—		Mean index
	1938	1939	
2-inch straw mulch	3.0	4.0	3.5
Soybean shade	38.0		38.0
No mulch (weeded only)	48.0	36.5	42.2
Soil mulch (cultivation)	43.0	45.0	44.0
Muck-soil mulch	52.0		52.0
Alfalfa as green manure	68.0	46.5	57.2
Straw as organic matter		34.0	34.0

[1] Mean of 4 replicates.

Soil management studies were continued in 1939 with the exception of the soybean shade and muck-soil mulch. One set of organic matter plots was added, however, in the form of rye straw plowed under. It was thought possible that the low necrotic index recorded in 1938 where rye-straw mulch was applied could have been due to the action of some constituent in the straw in addition to the effect of reduced temperature and the maintenance of a higher, more uniform soil moisture. A significant reduction in internal tuber necrosis again occurred only in the case of the straw mulch (table 4). The fact that the rye straw as organic matter showed no benefit would indicate that the effect of straw mulch is only one of reducing soil temperature and retaining a higher level of soil moisture.

In 1938 soil temperatures were recorded twice weekly with a thermocouple at 3-inch and 6-inch soil depths during July, August, and September in all the plots. Although the biweekly records were somewhat inadequate, considerable variations were recorded in soil temperature as a result of differences in soil management. The greatest temperature differences recorded were those between the straw-mulch and the no-mulch plots. The mean soil temperature under the straw

mulch was lower by 12.1° F. at the 3-inch level and 10.8° at the 6-inch level than at corresponding depths in the no-mulch plots.

Continuous soil temperature records were also taken in 1938 at 1½-inch, 3-inch, and 6-inch soil depths in the no-mulch cultivated field plot. Recording thermometers were housed in shelter boxes about 15 inches above the soil surface with the thermometer bulbs buried in a horizontal position in the row at the desired depth, 6 to 8 feet away from the shelter so as to avoid any shading or radiation effect from it. A continuous air temperature record was also taken. The number of hours above 85° F. at 1½-inch, 3-inch, and 6-inch soil levels and in the air are given in table 5. It will be seen that there is a very wide difference in degree hours between the different soil depths, ranging from 227 hours at the 1½-inch depth to 110 hours at the 6-inch depth; 117 degree hours were recorded for the air temperature.

TABLE 5.—*Total weekly hours when temperatures were above 85° F. in the air and at 1½-inch, 3-inch, and 6-inch soil depths in a nonmulched, cultivated field plot, in 1938 on Plainfield sand at Hancock, Wis.*

Week ending—	Hours of record	Hours above 85° F.				Weekly precipitation
		1½-inch depth	3-inch depth	6-inch depth	Air at soil level	
						Inches
July 24	168	50	18	10	11	0.44
July 31	168	31	19	12	13	.68
August 7	168	55	49	46	45	.75
August 14	168	37	23	20	21	.60
August 21	168	21	11	8	11	1.64
August 28	168	29	22	14	12	.38
September 4	[1]	4	8	0	4	.06
September 11	168	0	0	0	0	6.75
September 18	168	0	0	0	0	4.96
9-week total	1,512	227	150	110	117	15.26

[1] 120-week hours recorded.

Continuous temperature records obtained at 3-inch soil depth in nonmulched and straw-mulched field plots in 1939 and 1941 showed marked differences in soil temperatures. The 10-day air, soil, and rainfall records in table 6 were taken from field record sheets of July 23 to August 1, 1939, and July 24 to August 2, 1941. The mean soil temperature at 3-inch soil depth for the 10-day period in 1939 was 83.1° F. (nonmulched) and 74.2° (mulched) with a mean air temperature of 76.5°, and in 1941, 83.3° (nonmulched) and 70.0° (mulched) with a mean air temperature of 81.7°. The greatest difference in soil temperature, 26°, was recorded on July 24 and 31, 1941; however, 25° differences between nonmulched and mulched plots were not uncommon.

Although the application of the straw mulch was a means of reducing soil temperature, no direct evidence was secured which would indicate that the lower temperatures were more important in the reduction of internal necrosis than the conservation or uniformity of soil moisture. It is not possible to separate accurately the effect of soil temperature from that of soil moisture in field studies of this type.

TABLE 6.—*Air temperature record and soil temperature differences between nonmulched and straw-mulched potato field plots at 3-inch soil depth, Hancock, Wis., 1939 and 1941*

JULY 23 TO AUGUST 2, INCLUSIVE, 1939

Date	Air temperature		Soil temperature, 3-inch depth				Precipitation
			Nonmulched		Straw mulch		
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	
	°F.	°F.	°F.	°F.	°F.	°F.	*Inch*
July 23	91	55	96	72	78	66	
24	94	64	94	74	69	70	
25	91	70	93	77	78	72	
26	98	65	99	77	81	72	
27	97	65	100	81	82	74	
28	87	62	82	74	75	70	
29	81	50	86	65	75	68	0.97
30	86	55	91	66	77	68	
31	97	64	95	72	79	70	
August 1	93	65	95	75	79	72	
Average or total	91.5	61.5	93.1	73.3	78.3	70.2	.97
Mean	76.5		83.1		74.2		

JULY 24 to AUGUST 2, INCLUSIVE, 1941

July 24	98	69	98	71	72	65	
25	98	66	94	70	72	65	
26	102	72	96	71	72	66	
27	97	73	91	75	72	67	
28	94	71	89	72	72	68	.58
29	100	76	96	72	74	70	
30	96	70	96	75	75	69	.10
31	88	69	101	71	75	69	
August 1	85	69	95	70	72	68	
2	91	56	98	66	73	65	
Average or total	94.3	69.1	95.4	71.3	72.9	67.2	.68
Mean	81.7		83.3		70.0		

No doubt a disturbance of water balance within the tubers in the form of diminished respiratory activity and altered cell growth plays an important role in the occurrence and severity of internal tuber necrosis. Tubers grown under straw mulch do not produce the knobby apical outgrowths or growth cracks common in tubers from the no-mulch plots and the culinary quality of straw-mulched tubers is very much improved, the tubers being more mealy in texture, of better flavor, and with much less tendency to discolor after boiling even after 5 months in storage.[5]

When mulched and unmulched tuber lots were held in a common storage for 5 months, tubers from the unmulched lot were much more shriveled and sprouted very much earlier than those from the mulched lot.

Soil Treatment

Various soil, fertilizer, and amendment treatments were conducted on Plainfield sand at Hancock in 1938 and 1939. These treatments included the application of hydrated lime and sulfur to modify the soil reaction; combinations of macroelements in the form of nitrogen,

[5] The writers are indebted to the late Dr. W. E. Tottingham, Department of Biochemistry, University of Wisconsin, for making these tests.

phosphorus, and potash; microelements as soluble salts of boron, copper, iron, magnesium, manganese, and zinc incorporated in a complete fertilizer; and borax without fertilizer. In 1939 and 1940 soil treatments included various amounts of microelements alone in the form of salts of copper, manganese, and zinc. The initial soil applications were broadcast and disked in thoroughly on plowed ground about a month before planting. Plot planting, seasonal culture, and insect and foliage disease control were essentially the same as in the soil-management series.

The influence of various soil treatments on the incidence of tuber necrosis as expressed by the disease index is shown in table 7. The

TABLE 7.—*Soil treatment in relation to internal tuber necrosis*

Soil treatment	Pounds per acre	Disease index [1]—			Mean index
		1938	1939	1940	
Sulfur	1,000	35	38		36.5
0-62-0 fertilizer	200	39	37		38.0
Hydrate lime	2,000	49	36		42.5
8-8-15 fertilizer	800	58	29		43.5
Do.[2]	500	55	46		50.5
0-0-50	800	62	43		52.5
Borax	40	56	52		54.0
No treatment		48	43		46.0
Zinc sulfate	50		40	35	37.5
Do	100		42	32	37.0
Do	200		44	32	38.0
Manganese sulfate	100		45	34	39.5
Do	200		38	32	35.0
Do	300		38	31	34.5
Copper sulfate	100		50	38	44.0
Do	200		46	34	40.0
Do	300		41	31	36.0
No treatment			45	38	47.0

[1] Mean of 4 replicates.
[2] With additional microelements in the form of soluble salts of B, Cu, Fe, Mg, Mn, and Zn.

adjustment of soil reaction by the addition of hydrated lime and sulfur was readily secured. The initial soil reaction of about pH 5.5 was increased with the addition of 2,000 pounds per acre of hydrated lime to about 7.9 and decreased to pH 4.0 after the application of 1,000 pounds of sulfur. No significant difference in the incidence of tuber necrosis resulted at either pH. However, a definite variation in the severity of internal necrosis was noted when the cut tubers from the two ranges of soil pH were compared. Few tubers produced on the sulfur treated plots (pH 4.0) developed the severe internal necrosis of class 4 (fig. 2, *C*), whereas in the high alkaline soil (pH 7.9) a large percentage of this class was recorded. Partial control by various soil and amendment treatments have been reported by other workers but results have not been consistently successful (*15*, *16*, *19*, *21*).

DISCUSSION

Internal tuber necrosis is an important factor in commercial potato production during certain seasons in the sandy soil areas of central Wisconsin and in many instances has been a limiting factor in the production of a marketable crop. Because of the fact that there is a lack of distinguishable external tuber symptoms of the malady it is

not ordinarily detected until tubers are cut in the final culinary stage, in the dicing stage of dehydration, or sliced in chip manufacture.

In the present investigation, while an attempt was made to correlate the occurrence of internal tuber necrosis with the more variable environmental soil factors, such as temperature and moisture, and the more stable factors, such as the soil type, organic matter, fertility, and soil reaction, only the gross differences in the soil mass were measurable. The conditions immediately surrounding the potato tubers and roots undoubtedly exert an influence on the occurrence of internal necrosis but are inadequately measured by present methods.

The incidence of internal tuber necrosis is significantly different at successive periods in the development of tubers, the necrotic index in late-harvested tubers being more than double that of the earliest. Tubers of the Katahdin variety harvested 73 days after planting showed a necrotic index of 15.5, exhibiting only the slight and moderate diffused type of necrosis, whereas after 122 days the necrotic index had increased to 39.5 with more irregular, large, and concentrated lesions present. This difference, together with the fact that the amount and degree of necrosis is more severe in larger tubers, indicates that the malady has its inception during the initial tuber development and increases during growth of the tubers. There is no increase in the incidence of internal necrosis during storage.

In relation to the tuber depth in the soil, approximately the same incidence of internal necrosis was observed at the respective depths studied over a period of five seasons. The mean necrotic index in tubers exposed to 1 inch of soil cover was 40.72, with 1 inch to 2 inches of soil cover 15.17, and in tubers with over 2 inches of soil cover 0.49. These differences may explain in part, at least, the high incidence of internal necrosis in certain varieties which have a tendency to set tubers near the soil surface. Green end tubers did not show a greater incidence or degree of internal necrosis than the tubers with 1 inch of soil cover.

Considerable variation in the relative susceptibility of the varieties tested was observed. The standard varieties most resistant to internal necrosis were Triumph, Houma, Pontiac, and Red Warba, with indexes of 0, 1.7, 4.4, and 9.2, respectively; Rural New Yorker, Katahdin, Russet Rural, and Harmony Beauty with indexes of 39.2, 39.5, 40.5, and 54.5, respectively, were the most susceptible. It is of interest to note that Pontiac and Red Warba, both having Triumph as one parent, are among the most resistant. Field trials are now under way to study possible inheritance in seedling crosses. The Chippewa and Sebago varieties, replacing the Russet Rural as late-maturing varieties in the central Wisconsin area, are about equal in their susceptibility to internal necrosis, being 19.3 and 19.8, respectively. The Sebago variety, however, is gaining in preference over the Chippewa as a late variety because, in addition to foliage and tuber tolerance to late blight it has a very high field resistance to the yellow dwarf virus. All varieties under test, planted as an early crop (late April or early May) at Hancock, were free from internal necrosis.

In soil-management studies, straw mulch had a definite, beneficial effect in reducing internal necrosis in all field tests. No direct evidence was secured, however, that would indicate that a reduction in soil temperature was more important than the conservation or uniformity

of soil moisture. It is quite possible that in the unmulched soil a lack of proper water balance causing respiratory disturbances within the tubers during their early formation, altering cell growth and development, play an important role in the occurrence and severity of internal necrosis. The evidence obtained in limited field tests appears to indicate that in lighter soils the fluctuating factors of soil temperature and moisture are of greater significance than differences in organic-matter content, soil reaction, or soil fertility.

SUMMARY

The symptoms of physiological internal necrosis occurring in potato tubers produced on the lighter soils under central Wisconsin conditions are discussed.

Anatomical symptoms of necrotic lesions usually are first noted as a slight darkening of the corners, walls, and protoplast of the cells in the parenchymatous tissues of the internal medulla. The intercellular substance is also somewhat darkened. This is followed by the accumulation of protoplasm against the cell walls with intensified thickening and discoloration of the walls. Abnormal division of adjacent cells tends to reduce the collapsed cells to specific necrotic areas.

Microchemical studies showed internal necrosis to be associated with suberization in all cases where this type of tissue was found. The presence of pentoses was indicated. No definite indication of the accumulation of lignin, cellulose, or solanine in the necrotic tissue was evident. Starch grains were lacking in cells composing the affected areas and in contiguous tissues.

The study of seasonal development and tuber depth in the soil showed that necrosis increased both in amount and severity as the season advanced and that tubers with 2 inches or more of soil cover were very much less affected than those more lightly covered. No increase of internal necrosis was observed in tubers during storage.

The standard potato varieties, Triumph, Pontiac, and Red Warba, are much more resistant to the malady than are Katahdin, Rural New Yorker, Russet Rural, or Harmony Beauty.

Soil management in the form of straw mulch consistently reduced internal tuber necrosis. The application of hydrated lime, sulfur, combinations of macroelements, microelements as soluble salts of boron, copper, iron, magnesium, manganese, and zinc alone or incorporated in a complete fertilizer, and borax without fertilizer, did not reduce the incidence of internal necrosis.

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URINARY 17-KETOSTEROID EXCRETION BY BOARS [1]

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INTRODUCTION

Lines of inbred swine now being developed at the Minnesota Agricultural Experiment Station (*15*)[3] differ in the rate of sexual development and libido of the boars and the time of first estrus of the females. In the Minn. No. 1 line the boars began ranting early in the fifth month of age. The ranting was quite pronounced and apparently affected their rate of gain (*14*). While these boars seldom lacked in sexual desire, they did not exhibit marked secondary sexual characteristics. In other lines, especially the Market Lady and C_2, the boars looked and acted more or less like barrows until 200 to 250 days of age. Later they frequently lacked in sexual interest but, in contrast to the Minn. No. 1 boars, they developed strong secondary sexual characters such as heavy shoulder shields.

Urinary 17-ketosteroids were assayed from boars of various lines in order to find "normal" excretory values for various ages, possible differences among lines, and the association between 17-ketosteroids and sexual behavior.

REVIEW OF LITERATURE

Androgenic substances have been recovered from boar testes by Ogata and Hirano (*12*) and Hanes and Hooker (*3*). The last-named authors found that normal testes contain about twice the amount of androgens per gram of tissue as cryptorchid testes. Marsman (*9*) and DeReget, as quoted by Marsman, found, respectively, 90 and 134 mouse units of androgens per liter of boar urine. They also found 836 and 1,167 units of female hormone per liter of urine. Green et al. (*2*) assayed androgens of 48-hour urine samples of boars and reported a range of 0.21–2.09 capon units per sample. By the use of the Holtorff and Koch (*4*) technique they recovered 1,408–7,646 γ androsterone

[1] Received for publication June 8, 1944. Paper No. 2159, Scientific Journal Series of the Minnesota Agricultural Experiment Station. The animals used in this study were obtained from a project carried on in cooperation with the Regional Swine Breeding Laboratory, Ames, Iowa, Bureau of Animal Industry, U. S. Department of Agriculture.

[2] The authors wish to acknowledge their indebtedness to Dr. R. E. Comstock, formerly of this station, for assistance in the statistical analysis of the data. Assistance in certain phases of the work was rendered also by personnel of Work Projects Administration Official Project No. 165–1–71–124, Subproject 143, sponsored by the University of Minnesota in 1941.

[3] Italic numbers in parentheses refer to Literature Cited, p. 516.

equivalent of 17-ketosteroids from the 48-hour urine samples. They found a highly significant correlation of 0.88 between the results obtained by the capon comb and the Holtorff and Koch assay techniques. They also reported one 48-hour urine sample to contain 71.45 γ equivalent of estrone by the mouse vaginal smear technique.

METHODS

The methods for the collection of urine and the assay of 17-ketosteroids were the same as those described in an earlier report (*2*). The benzene used for extraction was thiophene-free and distilled before use. In order to standardize environmental conditions, boars representing the various lines were brought from the agricultural experiment substations to the laboratory immediately after weaning (8 weeks of age). They were kept in one pen so that all would have access to the same ration,[4] water supply, etc. For the urine collections, boars of similar ages were divided into groups of four, and the division was such that no two boars of the same line were in the same group. Collections were rotated so that each boar was sampled at 10- to 14-day intervals. The boars always remained in the collection crates exactly 48 hours. During that period they were fed a slop feed three times a day and watered twice daily. All results are given on the basis of 48-hour urine samples and are reported as γ equivalent of androsterone.[5] Statistical methods of Snedecor (*13*) were used for analysis of the data.

RESULTS

The benzene extracts possessed a red color similar to that of human urine extracts. Since some assays were completed before corrections were made for this residual color, studies were conducted to find whether the color had materially affected the results.[6] A nonsignificant correlation of −0.018 was found between the amount of residual color in an assay and the amount of 17-ketosteroid of that sample before correction. Correlation coefficients between the amount of urine per sample and the amount of 17-ketosteroids of samples (a) corrected or (b) uncorrected for color were not significantly different (185 samples). Since the amount of residual red color did not appear to affect the reliability of the results, all data reported hereafter are on the "uncorrected" basis.

EFFECT OF AGE AND SEASON OF THE YEAR ON 17-KETOSTEROID EXCRETION

The amount of 17-ketosteroids excreted varied with the age of the boars (fig. 1 and tables 1–3). Season of the year apparently affected excretion in two ways: (1) The season of the year in which the animals were farrowed, and (2) seasonal effects, as such, independent of the season of farrow. These latter seasonal effects appeared

[4] Whenever buttermilk was fed as a portion of the protein supplement the urine foamed excessively when the HCl was added for hydrolysis, and this resulted in the loss of a number of samples. Only quantitatively recovered samples were used for assay.

[5] The authors are indebted to Dr. Erwin Schwenk, of the Shering Corporation, for supplying androsterone for colorimeter standardization.

[6] All extracts were brought to a 25-ml. volume after separation of the female hormone fraction and 0.2 ml. of the 25 ml. were used for each assay tube.

to be exerted even though the boars were of different ages at a specific calendar period.

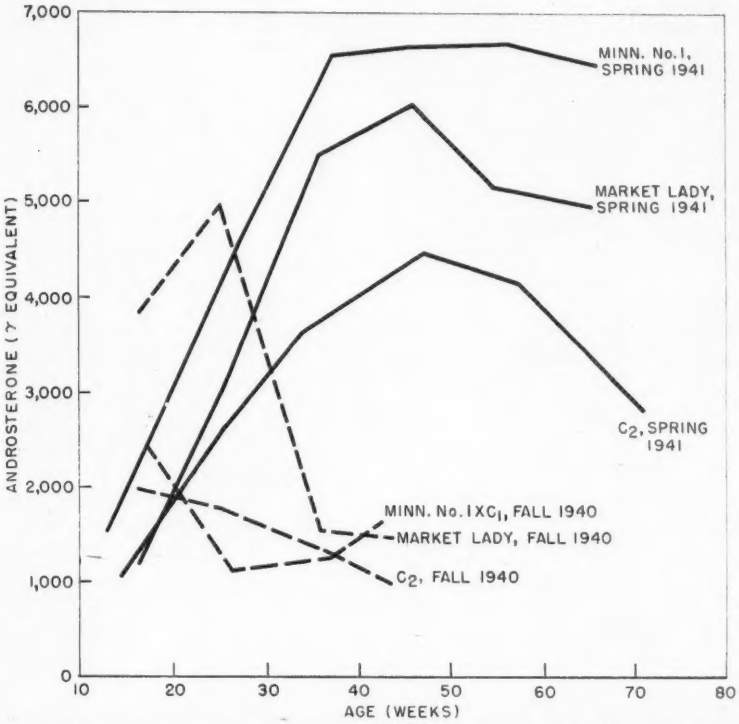

FIGURE 1.—Average 17-ketosteroid excretion during age periods indicated of boars of different ages, line of breeding, and season of farrow.

TABLE 1.—*Regression coefficients of 17-ketosteroid (y) on age (x) for the lines, ages, and time of farrow of fall- and spring-farrowed boars*

Fall-farrowed boars			Spring-farrowed boars		
Age in weeks	Line	b_{yx}	Age in weeks	Line	b_{yx}
10–20	C_2	106	10–20	C_2	51
	Market Lady	388		Market Lady	88
	Minn. No. 1×C_1	208		Minn. No. 1	319**
21–30	C_2	−207**	21–30	C_2	309*
	Market Lady	−362		Market Lady	268**
	Minn. No. 1×C_1	−19		Minn. No. 1	182
31–40	C_2	157**	31–40	C_2	379**
	Market Lady	−27		Market Lady	−164
	Minn. No. 1×C_1	−40		Minn. No. 1	104
41–50	C_2	−9	41–50	C_2	−7
	Market Lady	−187		Market Lady	29
	Minn. No. 1×C_1	402*		Minn. No. 1	−525*

*Statistically significant at the 5-percent level.
**Statistically significant at the 1-percent level.

TABLE 2.—*17-Ketosteroid excretion of spring-farrowed boars by age and line of breeding*

Age in weeks	Line	Number of boars used	Number of samples	$\bar{s}$ [1]	$\bar{x}$ [2]	$\bar{y}$ [3]	Ratio $\bar{y}$ to $\bar{u}$ [4]
10–20	C_2	4	15	9.2	14.2	1,052	0.369
	Market Lady	4	14	24.8	16.0	1,199	.406
	Minn. No. 1	6	21	20.3	13.0	1,537	.349
	N. W. Black Star	1	1	21.0	13.0	1,084	.847
	All lines, total or average	15	51	18.3	14.8	1,293	.371
21–30	C_2	3	14	25.1	25.5	2,642	.576
	Market Lady	4	22	37.6	25.6	3,106	.906
	Minn. No. 1	3	12	39.7	27.0	4,550	1.036
	N. W. Black Star	1	2	40.5	29.5	5,976	1.594
	S. E	1	2	42.0	28.0	4,572	1.514
	C_1	2	2	30.0	26.0	2,130	1.910
	All lines, total or average	14	54	34.8	26.2	3,432	.781
31–40	C_2	2	8	36.9	34.0	3,636	1.136
	Market Lady	4	14	51.9	35.7	5,502	1.551
	Minn. No. 1	3	9	54.0	37.2	6,560	1.401
	N. W. Black Star	1	1	44.0	32.0	8,950	6.393
	S. E	1	2	56.5	38.0	4,872	1.686
	C_1	4	10	42.3	35.7	2,614	2.213
	All lines, total or average	15	44	47.4	35.8	4,772	1.539
41–50	C_2	2	5	55.6	47.2	4,483	1.657
	Market Lady	3	12	65.0	45.9	6,030	2.282
	Minn. No. 1	4	10	66.1	45.3	6,629	1.922
	All lines, total or average	9	27	63.7	45.9	5,965	2.020
51–60	C_2	1	2	69.0	57.0	4,189	1.614
	Market Lady	3	10	76.8	54.7	5,165	2.500
	Minn. No. 1	3	6	80.7	56.0	6,676	1.457
	All lines, total or average	7	18	77.2	55.4	5,560	1.876
61–76	C_2	2	5	88.6	71.0	2,845	.937
	Market Lady	3	5	90.2	64.8	4,945	1.003
	Minn. No. 1	3	4	94.3	65.5	6,450	1.755
	All lines, total or average	8	14	90.8	67.1	4,625	1.195

[1] $\bar{s}$ = Average season unit designation: The year was divided into 5 day unit intervals with the result that unit No. 74 coincided in calendar dates, May 1–5, with unit No. 1.

[2] $\bar{x}$ = Average age in weeks.

[3] $\bar{y}$ = Average γ androsterone equivalent per sample.

[4] $\bar{u}$ = Average milliliters of urine per sample.

Figure 1 indicates that during the age period of 10–20 weeks the fall-farrowed boars excreted more hormones than the spring boars. An analysis of variance made separately for the Market Lady and C_2 lines showed that the fall boars did excrete significantly more than the spring-farrowed ones.

Since the effects of season and age were necessarily intermingled, an attempt was made to separate their respective influences. Study of the dissimilarity in the hormone excretion of fall- and spring-farrowed boars was made on all lines. The significance of this difference was tested by comparing, in an analysis of variance, the shifts during 2-month periods in 17-ketosteroid excretion of spring and fall boars of approximately the same age. For example, the 1940 fall boars averaged 14 weeks of age when December assays were made; the average change in their hormone excretion from December to February was compared with that from July to September of 1941 spring boars which averaged 14 weeks of age at the time the July

TABLE 3.—*17-Ketosteroid excretion of fall-farrowed boars by age and line of breeding*

Age in weeks	Line	Number of boars used	Number of samples	$\bar{s}$ [1]	$\bar{x}$ [2]	$\bar{y}$ [3]	Ratio of $\bar{y}$ to $\bar{u}$ [4]
10–20	C_2	2	12	49.9	16.0	1,990	0.396
	Market Lady	3	15	48.7	16.2	3,833	.663
	Minn. No. 1×C_1	2	13	56.1	16.9	2,439	.454
	All lines (total or average)	7	40	51.5	16.4	2,827	.521
21–30	C_2	2	11	62.6	25.0	1,787	.306
	Market Lady	3	16	57.8	25.2	4,968	.861
	Minn. No. 1×C_1	2	7	68.7	26.4	1,115	.213
	All lines (total or average)	7	34	61.6	25.4	3,145	.553
31–40	C_2	2	9	77.6	36.1	1,318	.283
	Market Lady	3	16	76.0	35.6	1,524	.245
	Minn. No. 1×C_1	2	10	82.6	36.4	1,267	.408
	All lines (total or average)	7	35	78.3	36.0	1,398	.284
41–50	C_2	2	6	88.3	43.6	999	.300
	Market Lady	3	8	82.7	43.7	1,495	.254
	Minn. No. 1×C_1	2	4	91.7	42.5	1,612	.292
	All lines (total or average)	7	18	86.8	43.4	1,356	.274

[1] $\bar{s}$=Average season unit designation.
[2] $\bar{x}$=Average age in weeks.
[3] $\bar{y}$=Average γ androsterone equivalent per sample.
[4] $\bar{u}$=Average milliliters of urine per sample.

TABLE 4.—*Summary of comparisons of shifts in hormone excretion by spring- and fall-farrowed boars during comparable age periods*

Fall-farrowed boars			Spring-farrowed boars			*F* value
Time interval	Boars	Change in excretion	Time interval	Boars	Change in excretion	
	Number	γ		*Number*	γ	
Dec. 1940–Feb. 1941	5	2,947	July 1941–Sept. 1941	7	1,130	7.36*
Jan. 1941–Mar. 1941	6	−3,338	Aug. 1941–Oct. 1941	8	1,577	41.00**
Feb. 1941–Apr. 1941	6	−2,786	Sept. 1941–Nov. 1941	7	2,099	19.81**
Mar. 1941–May 1941	6	883	Oct. 1941–Dec. 1941	8	2,577	11.28**
Apr. 1941–June 1941	6	507	Nov. 1941–Jan. 1942	9	1,954	14.194**
May 1941–July 1941	6	−467	Dec. 1941–Feb. 1942	8	473	.797
June 1941–Aug. 1941	5	207	Jan. 1942–Mar. 1942	5	−289	.36

*Significant *F* value at the 5-percent level.
**Significant *F* value at the 1-percent level.

assays were made. Comparisons were made for seven different age periods, each period overlapping the preceding one by 1 month. The essential data, recorded in table 4, show that the change in hormone excretion with age was significantly different for these fall and spring pigs, which suggests a seasonal effect.

The differences in quantity of 17-ketosteroid just noted were not due to the amount of urine per sample. Urine excretion was analyzed in a manner similar to that used for the alternate month comparisons in the excretion of hormone. In only one of the seven instances was the difference in urine quantity significant (at the 5-percent level), namely, that for the April-June and November-January data. Quantity of urine decreased while 17-ketosteroid increased; so amounts of urine were not related directly to hormone values.

Data for studying seasonal effects on the rate of 17-ketosteroid excretion were secured by comparing fall boars which were sampled from December 1940 through August 1941 and spring boars which were sampled from June 1941 through August 1942. There were, therefore, two groups of boars approximately 6 months apart in age sampled during the same months of the summer of 1941 and similar months of separate years.

Tables 2 and 3 show a striking similarity of the effect of season on 17-ketosteroid excretion. After s values of 50–63 all lines showed a consistent decrease in excretion except the Minn. No. 1 × C_1 crossbred group which rose slightly after an average s value of 68. Although the Market Lady and C_2 lines differed during the period of 50–60 s units, each line was consistent in that the trends for the fall- and spring-farrowed boars of a line were similar. When the data for the C_2 and Market Lady lines for both fall and spring boars were combined for the age periods of 21–30 and 41–50 weeks, respectively, the regression coefficient of 17-ketosteroid and age with seasonal units (effects) held constant ($b_{yx \cdot s}$) was 87 while the regression coefficient of hormone on season with age effects held constant ($b_{ys \cdot x}$) was −145, which indicated that age and season were exerting different effects on hormone excretion during that season of the year.

Differences in the amount of urine per sample would not necessarily account for the seasonal influence on 17-ketosteroid excretion, because season of the year had little effect on the trend of the quantity of urine per sample. Quantity of urine was closely associated with age and followed similar trends for both fall and spring pigs.

RELATION OF QUANTITY OF URINE TO 17-KETOSTEROID PER SAMPLE

As age and season of the year apparently affect 17-ketosteroid excretion and as all of the correlations of hormone and urine quantities were positive (table 5), and in some cases more highly significant than age and season correlations, a study was made to find the association between urine excretion and hormones.

In order to test whether age or quantity of urine was most closely correlated with hormone excretion, the method of comparing correlation coefficients given by Baten (*1*) was used in all cases where the independent variables, age and urine quantity, were significantly correlated. F values were calculated from regression data in cases where the independent variables were not significantly correlated. In the case of the fall boars of ages 10–20 weeks, the correlations were significantly different, but the correlation of hormone and age was greater than that of hormone and urine. In no case did the urine quantity predict the amount of 17-ketosteroid better than age.

Correlation or regression coefficients of hormone quantity and amount of urine per sample may be misleading in indicating the trend of 17-ketosteroid values. In the case of the spring-farrowed boars in the age groups 41–50 and 51–60 weeks, the correlation coefficients of hormone and age, urine and age, and hormone and season were negative (table 5), while the coefficient of hormone and urine are positive. As both hormone and urine quantities are decreasing with age, they would be positively associated if the data involving hormones and urine were plotted on a correlation surface.

TABLE 5.—*Correlation coefficients of 17-ketosteroid excretion (y) and age (x), urine quantity (u), and season index (s) for boars of different ages*

Age in weeks	r_{ys}	r_{yu}	r_{us}	r_{ys}
Spring-farrowed boars:				
10–20	0.445**	0.452**	0.374*	0.525**
21–30	.544**	.369*	−.016	.611**
31–40	.159	.308	−.114	.146
41–50	−.215	.775**	−.357	−.219
51–60	−.052	.672*	−.085	−.043
61–76	.825*	.779*	.755*	.761*
Fall-farrowed boars:				
10–20	.680**	.518**	.750**	.695**
21–30	−.341	.306	−.158	−.372
31–40	.088	.102	−.433*	.043
41–50	−.265	.212	.152	−.269

*Statistically significant at the 5-percent level.
**Statistically significant at the 1-percent level.

If the amount of 17-ketosteroids were dependent upon the amount of urine excretion, the ratio of hormone quantity to urine quantity should be constant. This ratio, however, increased from 0.37 to 2.02 for the spring-farrowed boars up to the age of 41–50 weeks (table 2) and decreased from 0.52 to 0.27 for the fall pigs of the same ages (table 3). The range in ratios was from 0.21 to 6.39 among the various lines and age groups. The lines of swine also varied in their hormone-urine ratios; the Market Lady spring boars had a higher ratio than the Minn. No. 1, which in turn had a higher ration than C_2 boars.

Further evidence of the lack of association between 17-ketosteroid excretion and urine quantity was shown by the fact that (1) trends in urine and hormone quantities were not similar when plotted against age; (2) that fall boars consistently excreted more urine on an average than spring boars but less hormone after 20 weeks of age; and (3) that the peak in the quantity of urine for both fall- and spring-farrowed boars occurred during the 21–30 week age period and 17-ketosteroid levels continued upward in the spring boars after that age.

These data seem to indicate that although the correlations between amount of 17-ketosteroid and amount of urine per sample were positive, and in some cases significantly so, these correlations are the result of circumstance and have little value in determining the actual trends of hormone excretion nor is the amount of 17-ketosteroid especially predicted by urine quantity.

LINE DIFFERENCES AND BOAR DIFFERENCES IN 17-KETOSTEROID EXCRETION

Boars of the Minn. No. 1 line farrowed in the spring consistently excreted more 17-ketosteroids than did spring-farrowed boars of the Market Lady or C_2 lines. In both fall and spring groups, the boars of the Market Lady line excreted more than the boars of the C_2 line (fig. 1 and tables 2 and 3). Data for the boars of other lines are given in tables 2 and 3 as a matter of interest, but the numbers are too small to permit definite conclusions.

Differences among the lines were not statistically significant at all ages. The degrees of freedom for an analysis of variance were keyed as follows: Differences between lines, among boars within lines, within boars within lines, and total.[7] Among the spring-farrowed boars, line differences, tested by boars within lines ($P = <0.01$), were found at

[7] *F* values for testing general line differences would result from testing the mean square of variance due to between lines by boars within lines. Line differences based only on the boars used in this experiment would result from testing between lines by within boars within lines.

21–30 weeks and line differences based only on the boars used were found at 31–40 weeks. Differences ($P=<0.05$) were indicated among boars within lines at 31–40 and 51–60 weeks. The fall pigs, 31–40 weeks, differed among the lines at the 5 percent level when lines were tested in the second manner. Variations among boars within lines ($P=<0.05$) were noted among the 10- to 20-week-old fall boars.

ANDROGEN AND 17-KETOSTEROID EXCRETION AFTER CASTRATION

A few stags were assayed by both the capon comb growth technique and the colorimetric method. Values for 17-ketosteroids were lower after castration although they were never absent. Results secured from one C_2 boar castrated at 325 days of age and assayed by the capon technique are shown in table 6.

DISCUSSION

The lines of swine used for this study are still undergoing development and are under constant change. As generations pass, variation in characteristics, such as degree of sex desire and earliness of sexual maturity, is continuous and problems prevailing during the years 1940 and 1941, although still present, appear in different intensities, in different lines, or in both. The results obtained in this study are based upon the conditions prevailing during the years the lines were sampled. Whether or not a second sampling of the lines would give the same results is, of course, open to question; however, that does not necessarily detract from the importance of the implications of this study.

The trend in the excretion of 17-ketosteroids by the spring-farrowed boars of different ages was similar in pattern to the excretion of androgens in human urine reported by Nathanson et al. (*10*), Neustadt and Myerson (*11*), and others. It was also similar to the change in the amount of androgens in bull testes of different ages (Hooker, *5*). The quantities of hormones excreted rose steadily from sometime before puberty until sometime afterwards. The decrease in excretion after a peak had been reached is similar to what occurs in bulls and in human beings. Since season was apparently exerting some effect about the time of the decline in hormone excretion, no definite conclusion could be reached as to whether the decline resulted from the influence of age or of season.

TABLE 6.—*Excretion of androgenic substances (measured in capon units) by a C_2 line boar before and after castration*

Dates of sample collection before castration	Androgenic substances	Date of castration	Dates of sample collection after castration	Androgenic substances
	Capon units			*Capon units*
August 21–23, 1940	1.64	November 23, 1940	January 13–15, 1941	0.39
September 25–27, 1940	1.08		January 20–22, 1941	0
			January 26–28, 1941	0

Two possible effects of season were noted; the spring-farrowed group excreted more 17-ketosteroids after 20 weeks of age than the fall-farrowed group. The excretion curves for the two groups were dissimilar, but those for all boars of one season's farrow tended to follow the same pattern. This indicated that some factor was exerting a similar influence on all the boars. Whether this was some phenomenon associated with the season of the year, or whether it was due to nutritional causes was not determined. All the boars were fed as nearly the same rations and treated as nearly alike as possible. In both spring and fall boars there was a reduced excretion of hormones in the latter part of February and early March. This was true even though the boars were 6 months apart in age and were sampled in different years. As the ration and management were constant throughout the year, it would seem reasonable to assign some of the effects observed to seasonal causes.

In view of this possible effect of season on 17-ketosteroid excretion, the data of Womack and Koch (*16*) are of interest. They found indications of possible seasonal differences in the androgen content of ram testes. It was slightly higher in August, October, November, and March than in January, February, and April. Rams will breed at any season of the year, but usually ewes will breed only in the fall. It may well be that seasonal changes in androgen production occur in the male of some farm animals even though it is not expressed in changes in the degree of sex drive.

The differences in the amount of 17-ketosteroid excreted by the various lines were not significant at all ages, but the consistency of the differences and the relative position of the lines is of considerable interest. With these animals, as with other species reported in the literature, the willingness of a particular individual to copulate was not necessarily correlated with sex hormone excretion. However, when the excretion of 17-ketosteroids was compared with the sex behavior of boars *by lines*, there seemed to be a rather close association between the two functions. As previously mentioned, the boars of the Minn. No. 1 line began to rant earlier and to a greater degree than the Market Lady or the C_2 boars. They also possessed more libido throughout life. In like manner the Market Lady boars preceded the C_2 boars in time and degree of sexual maturity as shown by sex drive. Differences among the lines in the age at which ranting or sexual desire appeared coincided quite well with the ages at which the regressions of hormone on age became significant as shown for the spring boars in table 1.

Differences among the lines in sex drive and response of somatic tissue is interesting in view of the work of Hooker (*6*), Hummel (*7*), and Kosin and Munro (*8*), who have shown that some tissues may be more responsive to androgenic stimulation at the time of puberty than before or after it, and that strains of mice and chicks showed a different response to pregnancy urine and androgens, respectively. The expressions of sex drive and of secondary sexual (somatic) characteristics in the Minn. No. 1 boars were the reverse of those in the Market Lady boars. Perhaps the ability of the somatic tissues and nervous system to respond to 17-ketosteroids at a given age varies among the lines in such a way that the response is not maximal at

the time of puberty in all cases. This might result in a lower response at a later age because of possible higher threshold-response values in older animals. On the other hand, the genetic constitution of the lines may affect the internal environment of the boars in such a way as to limit the response to 17-ketosteroids as well as determine the reactions at the time of puberty.

SUMMARY

Urinary 17-ketosteroid excretion by boars of different lines of inbred swine has been assayed from 48-hour urine samples. The residual red color of the benzene extracts was not found to have a material effect on the results of the assays. Correlations between the amount of urine per sample and 17-ketosteroids per sample were always positive and in some cases significant; however, the quantity of urine apparently had no real effect on the 17-ketosteroid values.

A sharp rise in 17-ketosteroid excretion at the time of puberty was not found. One stag excreted sufficient androgens to be assayed by the capon comb technique 58 days after castration but not 68 days after the operation.

Some effects of the season of the year on hormone excretion were noted.

The amount of 17-ketosteroids found in boars' urine varied with age and line of breeding. When compared on the basis of sexual behavior of the boars by lines, an association was found between the amount of hormone excreted and the sexual behavior of the boars. Some of the line differences in sex drive and somatic responses to the hormones were discussed.

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